Toxicity Profiling of Nanomaterials

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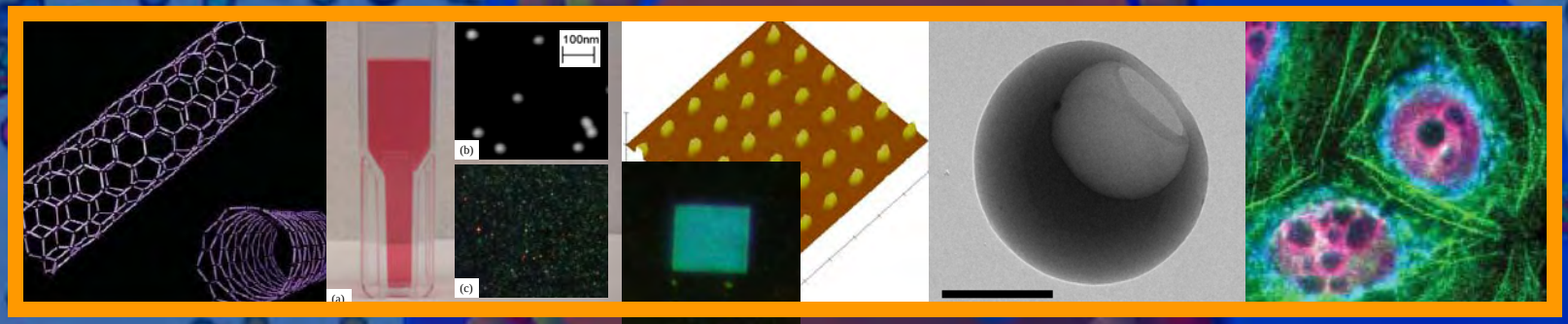
What is nanomaterial?

Nano – smaller and better

Nanomaterial is matter at dimensions of roughly 1~100 nm, where unique phenomena enable novel applications



- Optical, electromagnetic, mechanical enhancement
- Increasing stability or reactivity, smaller size, higher surface/mass ratio

The Scale of Things – Nanometers and More

Things Natural

Dust mite

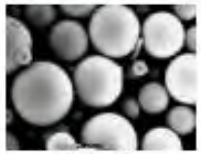
200 μm

Ant

~ 5 mm

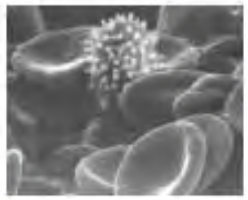
Human hair

~ 60-120 μm wide



Fly ash

~ 10-20 μm

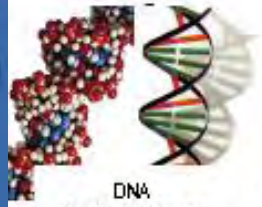


Red blood cells with white cell

~ 2-5 μm

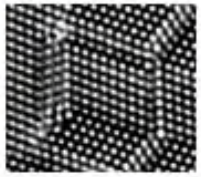
~10 nm diameter

ATPsynthase



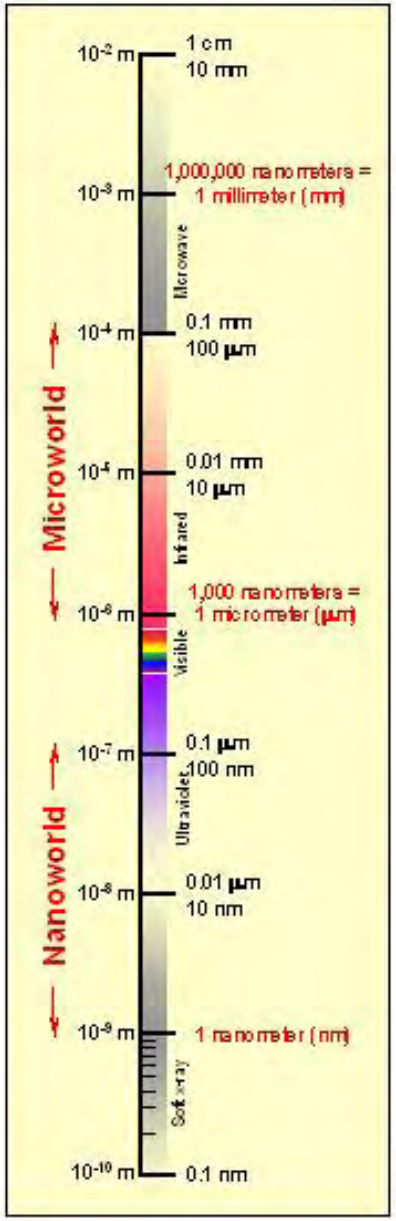
DNA

~2-12 nm diameter



Atoms of silicon

spacing ~tenths of nm



Things Manmade

Head of a pin

1-2 mm

MicroElectroMechanical (MEMS) devices

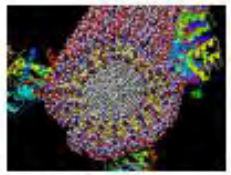
10 - 100 μm wide

Pollen grain

Red blood cells

Zone plate x-ray "lens"

Outer ring spacing ~35nm

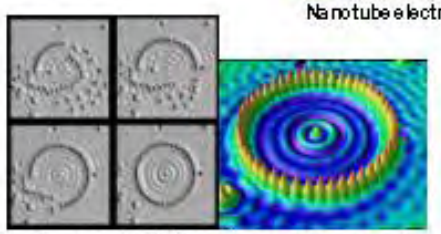


Self-assembled,

Nature-inspired structure

Many 10s of nm

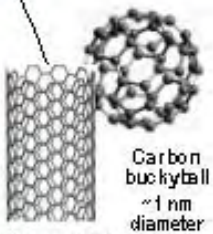
Nanotube electrode



Quantum corral of 48 iron atoms on copper surface

positioned one at a time with an STM tip

Corral diameter 14nm

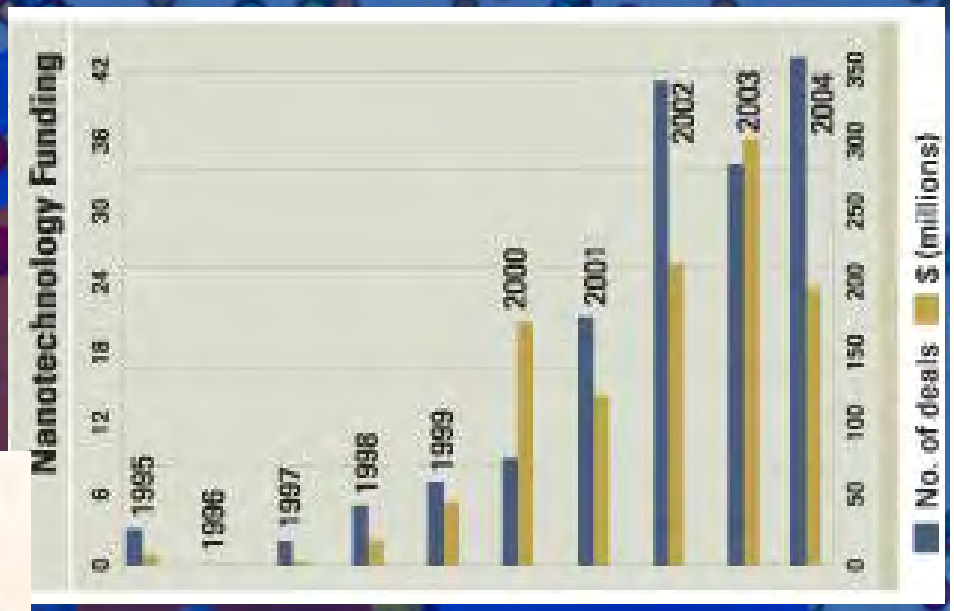


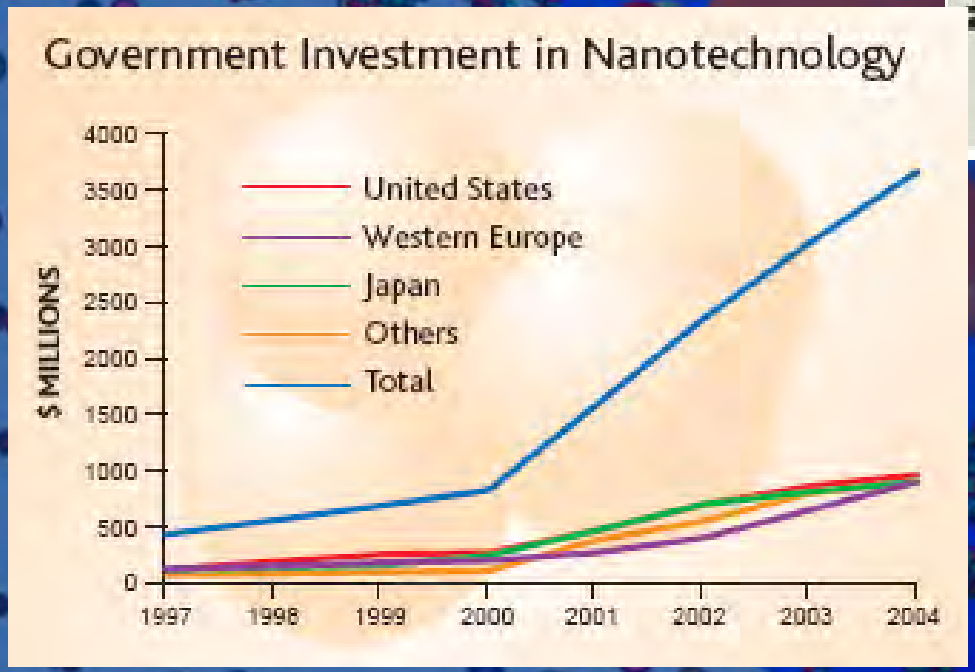
The Challenge

Fabricate and combine nanoscale building blocks to make useful devices, e.g., a photosynthetic reaction center with integral semiconductor storage.

BES, DOE

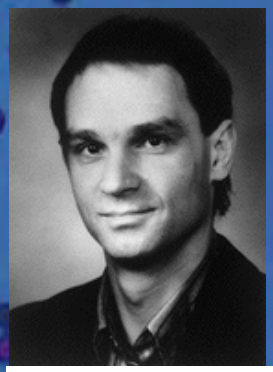
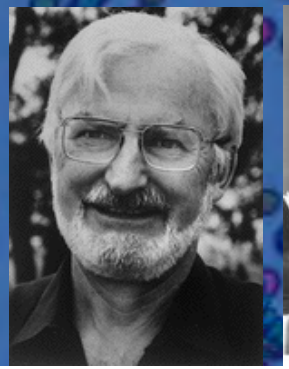
Government and Private Investments in Nanotechnology is skyrocketing



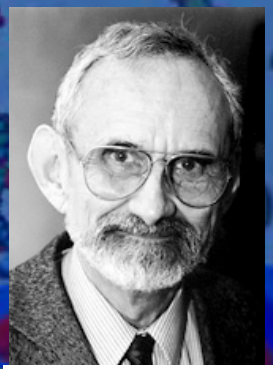
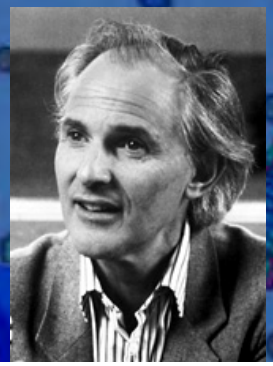
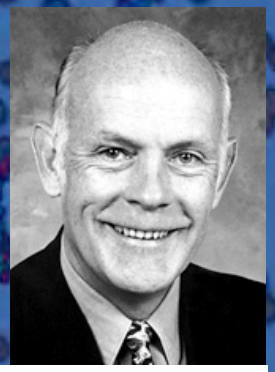


The coming nanoproduct flood

- >10,000 companies working on nanoproducts
- >50,000 products in R&D and commercial release pipeline
- Everyone is jumping onto the nanotechnology supertrain (medicine, engineering, research, environment, space, defense, homeland security, energy)


Nobel Prize, Phisics 1986
Ernst Ruska, Heinrich Rohrer, and Gerd Binnig

Nobel Prize, Chemistry, 1996
Robert F. Curl Jr., Sir Harold W. Kroto, and Richard E. Smalley

Nanotechnology is the new wave of technology innovation for the 21st century.

As nanoscience and nanotechnology come of age, the time for actively addressing the hazards associated with nanomaterials has arrived.

- Barnard AS. *Nature Materials* , 2006

Potential Health Hazards

- Extensive use of nanotechnology in biotech, pharmaceutical, chemical, and high-tech industries
- Solubilization, biocompatibilization, surface coating modifications
- Long-term persistence/stability
- Fast *in vivo* transportation
- Bioaccumulation
- Multiple entry routes, e.g. food (fish, plants, etc.), water, air entry routes)
- Cellular effects (stress responses, carcinogenesis, mutagenesis, cell cycle, cell death, differentiation, extracellular matrix, inflammation, DNA damage)

Toxic Warnings

1. 1997 - *Titanium dioxide/zinc oxide* nanoparticles from sunscreen are found to cause free radicals in skin cells, damaging DNA. (Oxford University and Montreal University) Dunford, Salinaro et al.
2. March 2002 – ... *engineered nanoparticles* accumulate in the organs of lab animals and are taken up by cells…“ Dr. Mark Wiesner
3. March 2003 - ... studies on effects of *nanotubes* on the lungs of rats produced more toxic response than quartz dust.“ „Scientists from DuPont Haskell laboratory present varying but still worrying findings on nanotube toxicity. Nanotubes can be highly toxic." - Dr. Robert Hunter (NASA researcher)
4. March 2003 - Dr. Howard: the smaller the particle, the higher its likely toxicity and that *nanoparticles* have various routes into the body and across membranes such as the blood brain barrier. ETC Group
5. July 2003 - Nature reports on work by CBEN scientist Mason Tomson that shows *buckyballs* can travel unhindered through the soil. "Unpublished studies by the team show that the nanoparticles could easily be absorbed by earthworms, possibly allowing them to move up the food-chain and reach humans" - Dr. Vicki Colvin, the Center's director.

Toxic Warnings

6. January 2004 - Dr. Günter Oberdörster: *nanoparticles* are able to move easily from the nasal passageway to the brain.
7. January 2004 - Nanosafety researchers from University of Leuven, Belgium in Nature: *nanoparticles* will require new toxicity tests: "We consider that producers of nanomaterials have a duty to provide relevant toxicity test results for any new material, according to prevailing international guidelines on risk assessment. Peter H. M. Hoet, Abderrrahim Nemmar and Benoit Nemery, University of Belgium(14)
8. January 2004 - Nanotox 2004: Dr. Vyvyan Howard presents initial findings that *gold nanoparticles* can move across the placenta from mother to fetus.
9. February 2004 - Scientists at University of California, San Diego discover that *cadmium selenide nanoparticles* (quantum dots) can break down in the human body potentially causing cadmium poisoning. "This is probably something the [research] community doesn't want to hear." - Mike Sailor, UC San Diego.(16)
10. March 2004 - Dr. Eva Oberdörster: *buckyballs (fullerenes)* cause brain damage in juvenile fish along with changes in gene function. "Given the rapid onset of brain damage, it is important to further test and assess the risks and benefits of this new technology before use becomes even more widespread." - Dr. Eva Oberdörster.

Environmental Concerns

- Can nanoparticles be released into the environment following human and animal use?
- What methodologies would identify the nature, and quantify the extent, of nanoparticle release in the environment?
- What might be the environmental impact on other species (animals, fish, plants, microorganisms)?

What's unique about Nanotoxicity????

- **Size matters!**

  penetration, clearance, intracellular accumulation different from larger particles

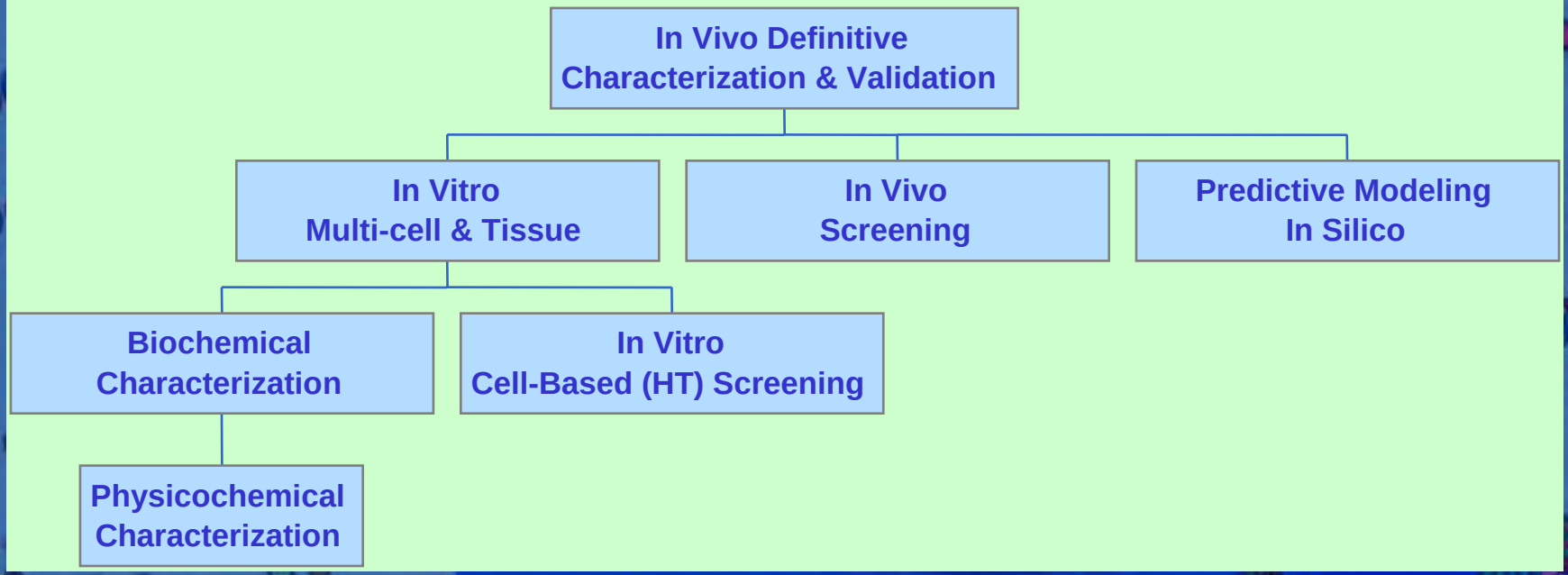
- **Surface**

- **Reactivity, structure rigidity**

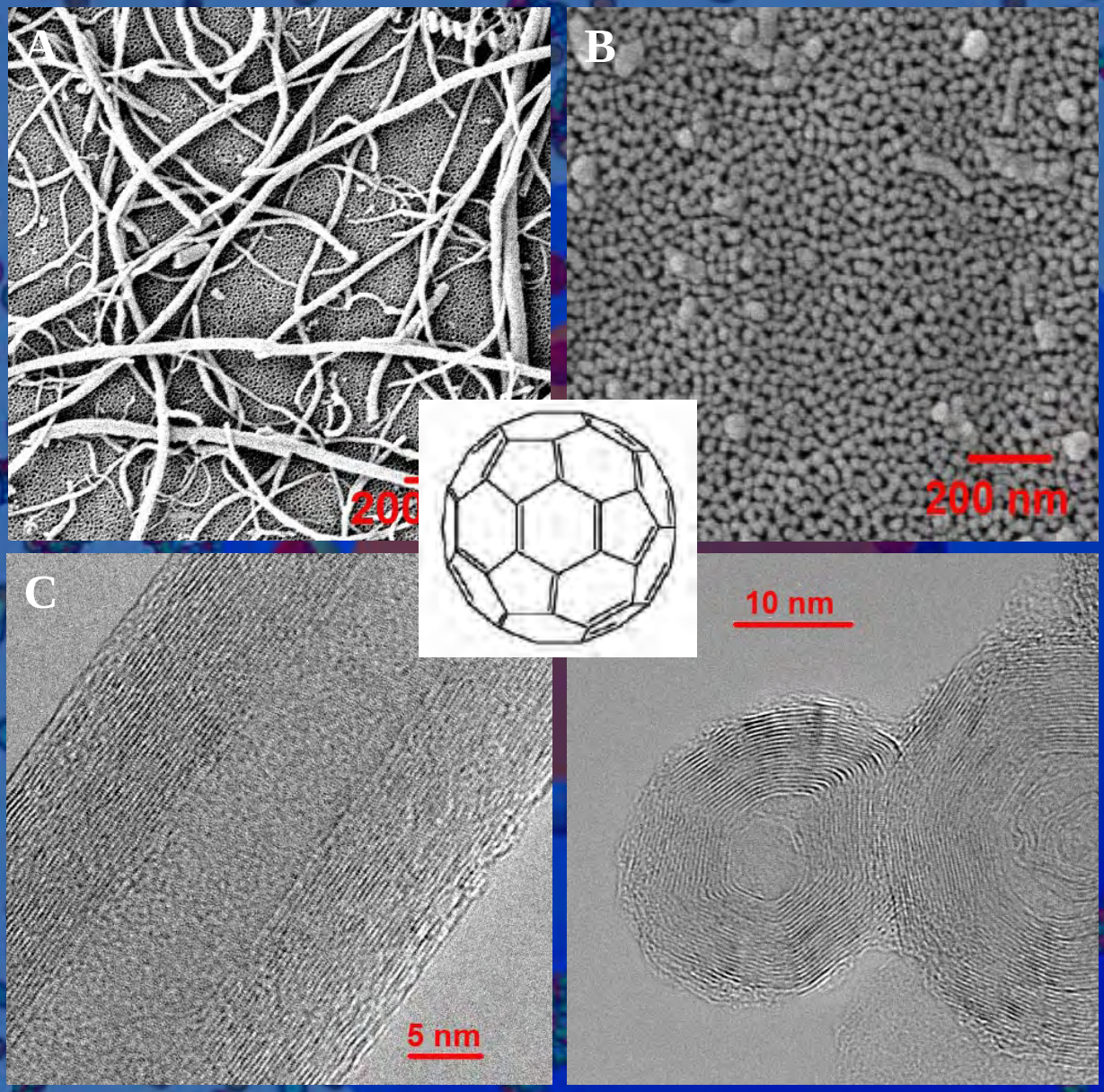
- **Detection, characterization, aggregation**

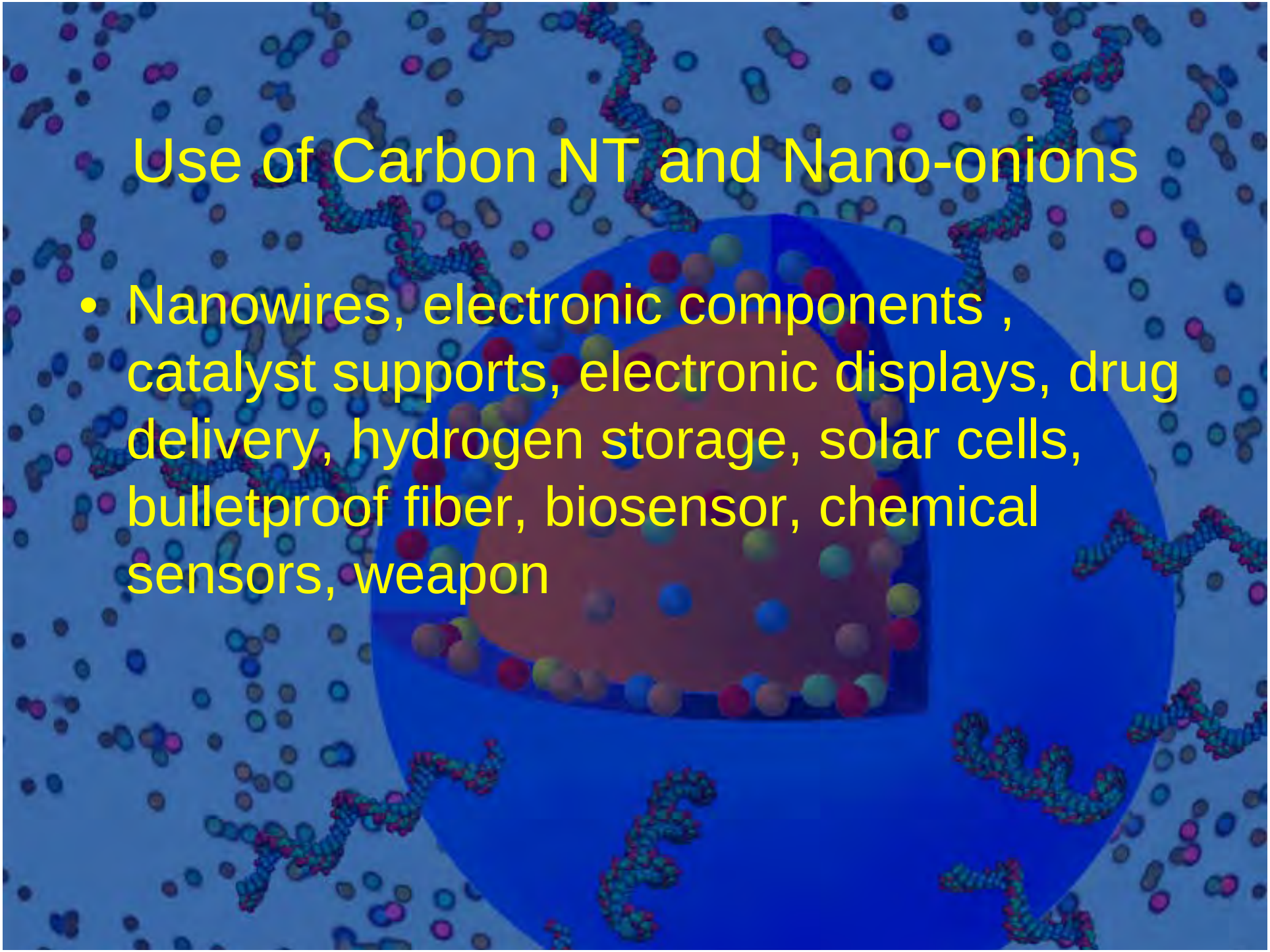
Six Tier Program

Decision Tree for Nanotechnology Safety Assessment



Nanotubes and Nano-onions

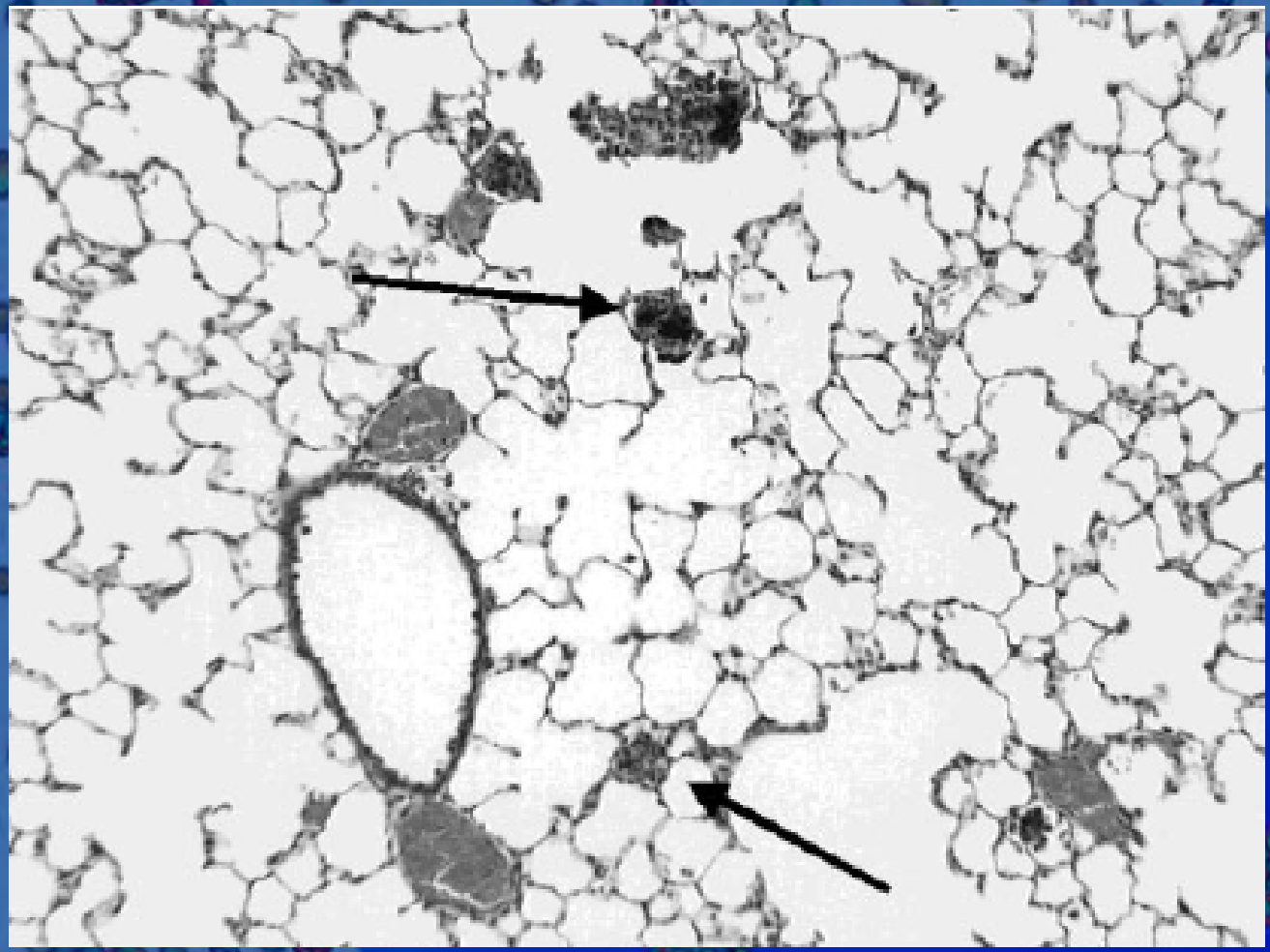




Use of Carbon NT and Nano-onions

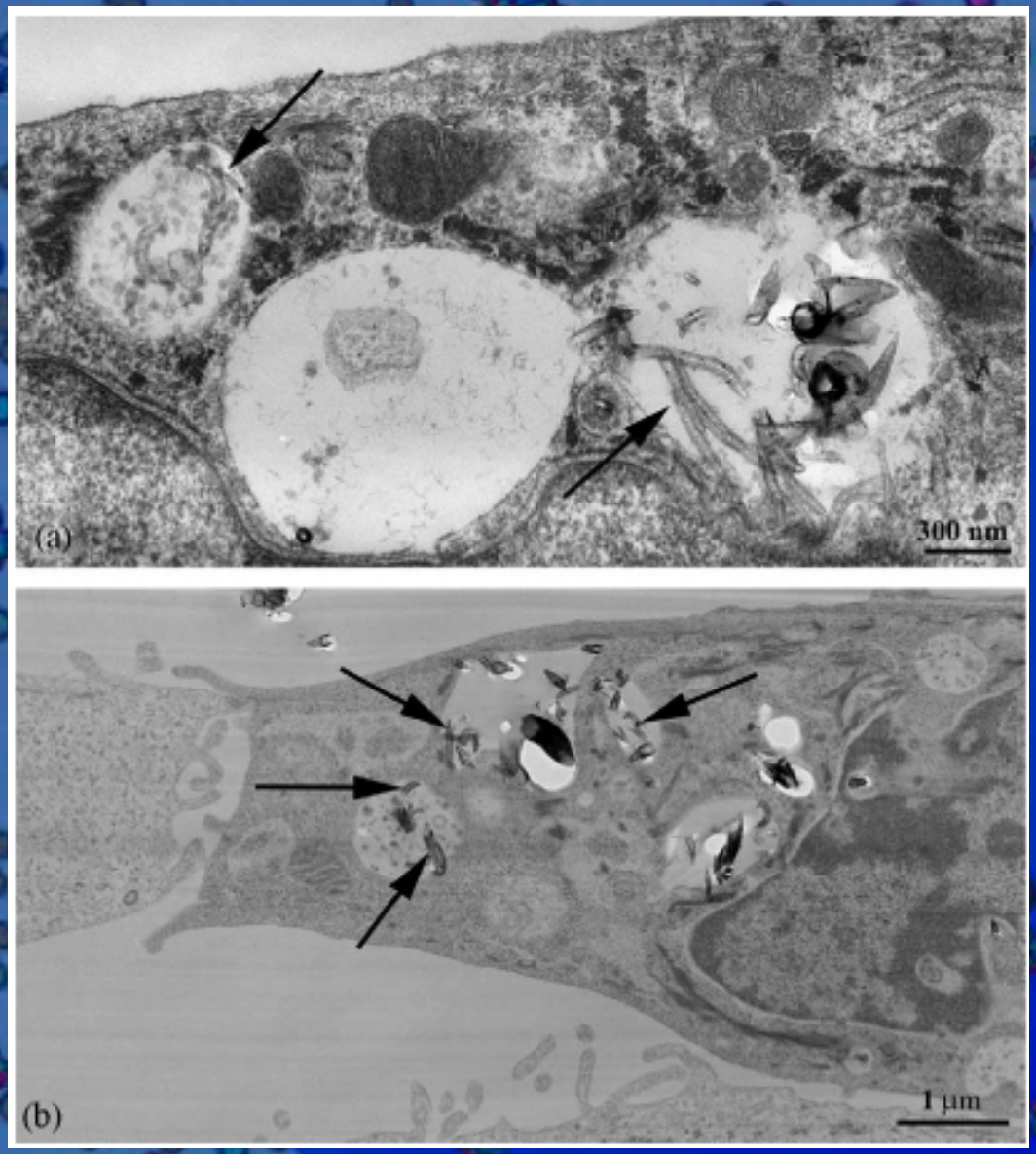
- Nanowires, electronic components , catalyst supports, electronic displays, drug delivery, hydrogen storage, solar cells, bulletproof fiber, biosensor, chemical sensors, weapon

Pulmonary toxicity by SWCNT



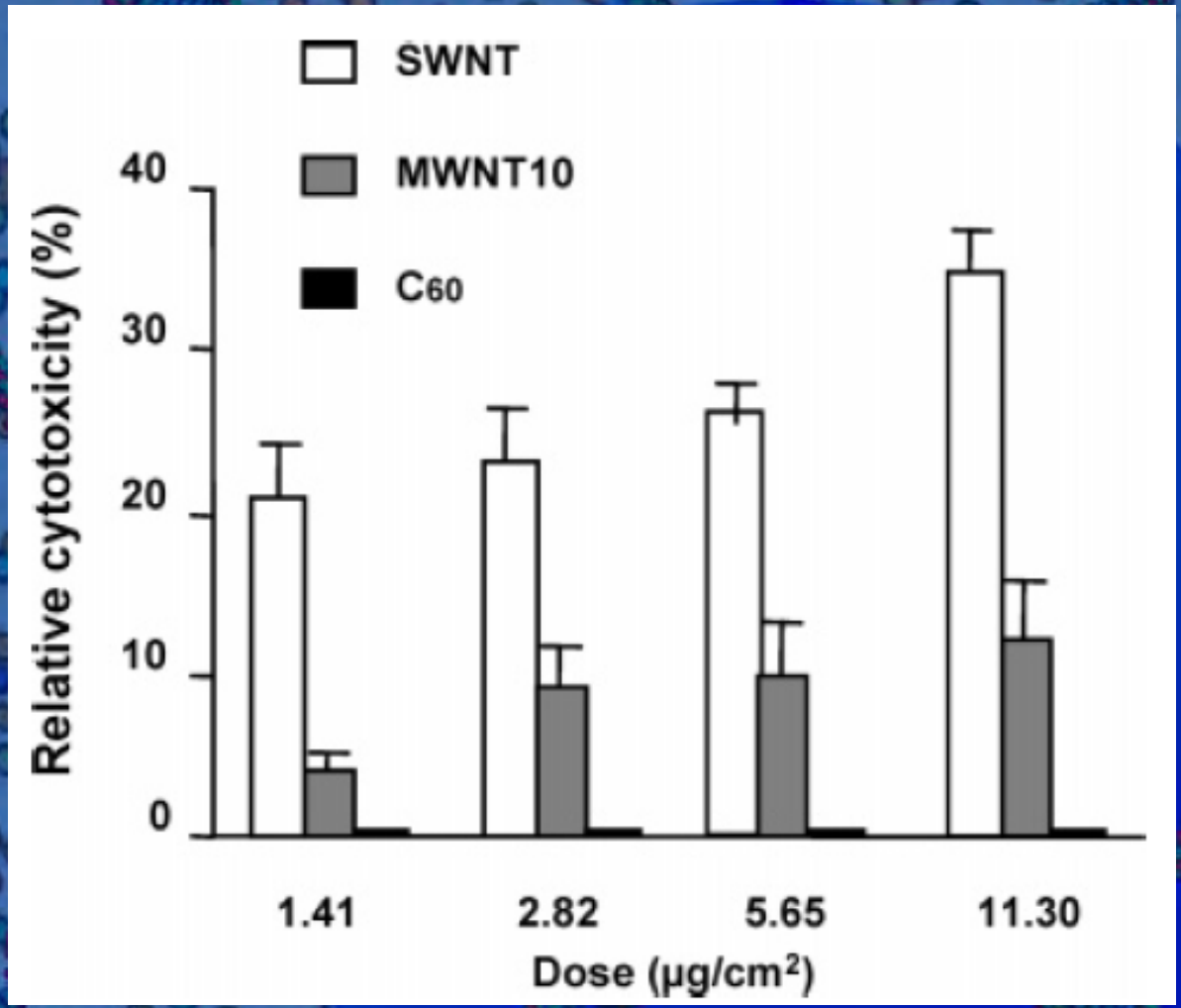
Warheit, D.B. et al. TOXICOLOGICAL SCIENCES ***77****, 117–125 (2004)*

Intracellular NT accumulation



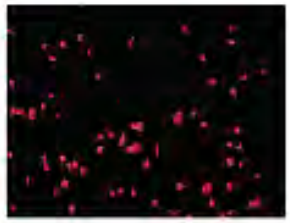
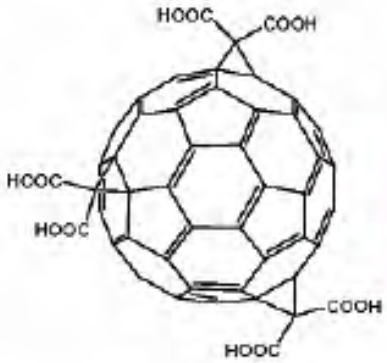
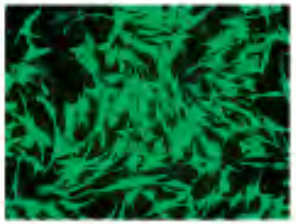
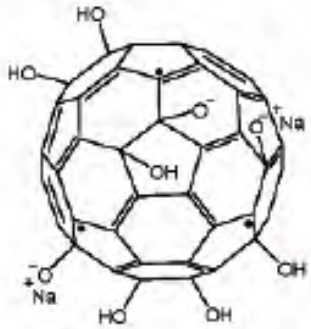
Monteiro-Riviere, N.A. Toxicology Letters 155 (2005) 377–384

NT cytotoxicity to aveolar macrophage



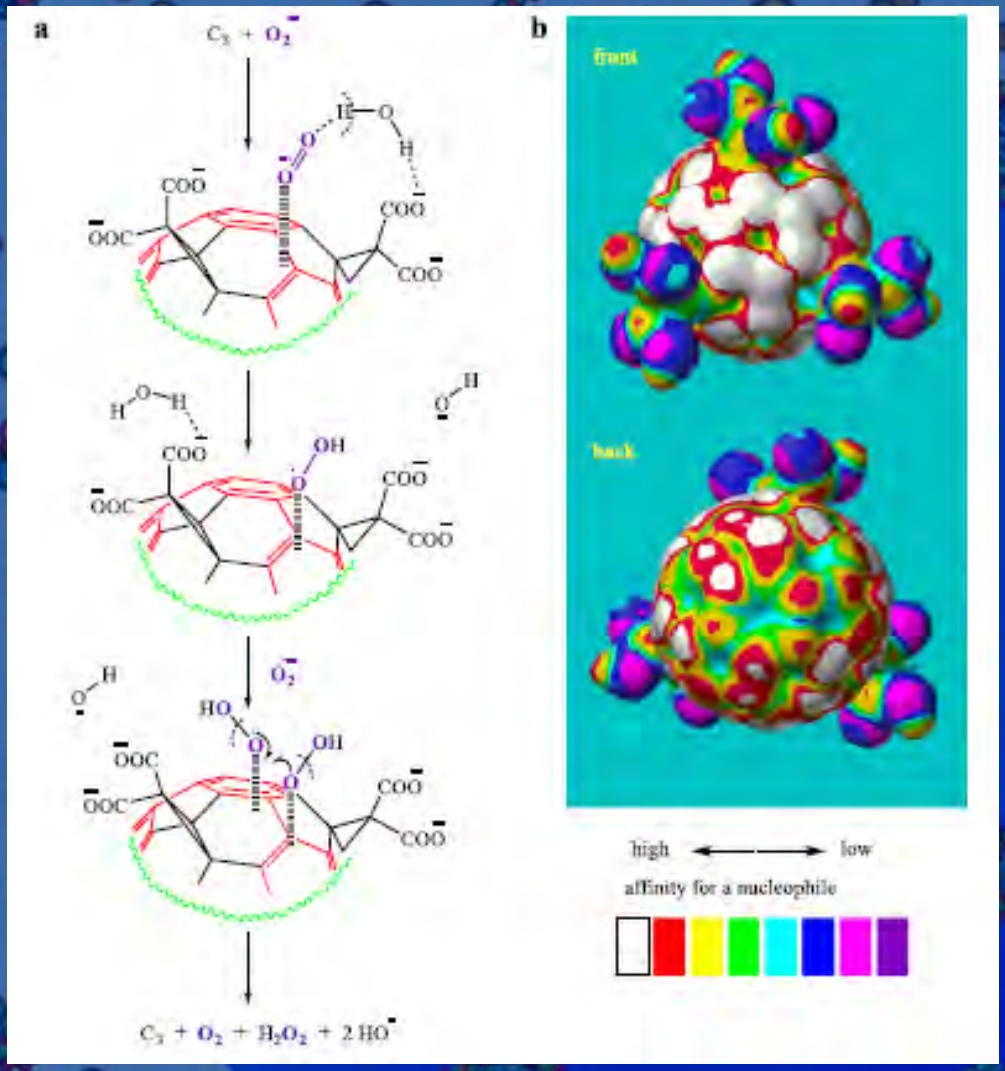
Jia, G. et al. Environ. Sci. Technol. ***2005****, 39, 1378-1383*

Fullerene cytotoxicity

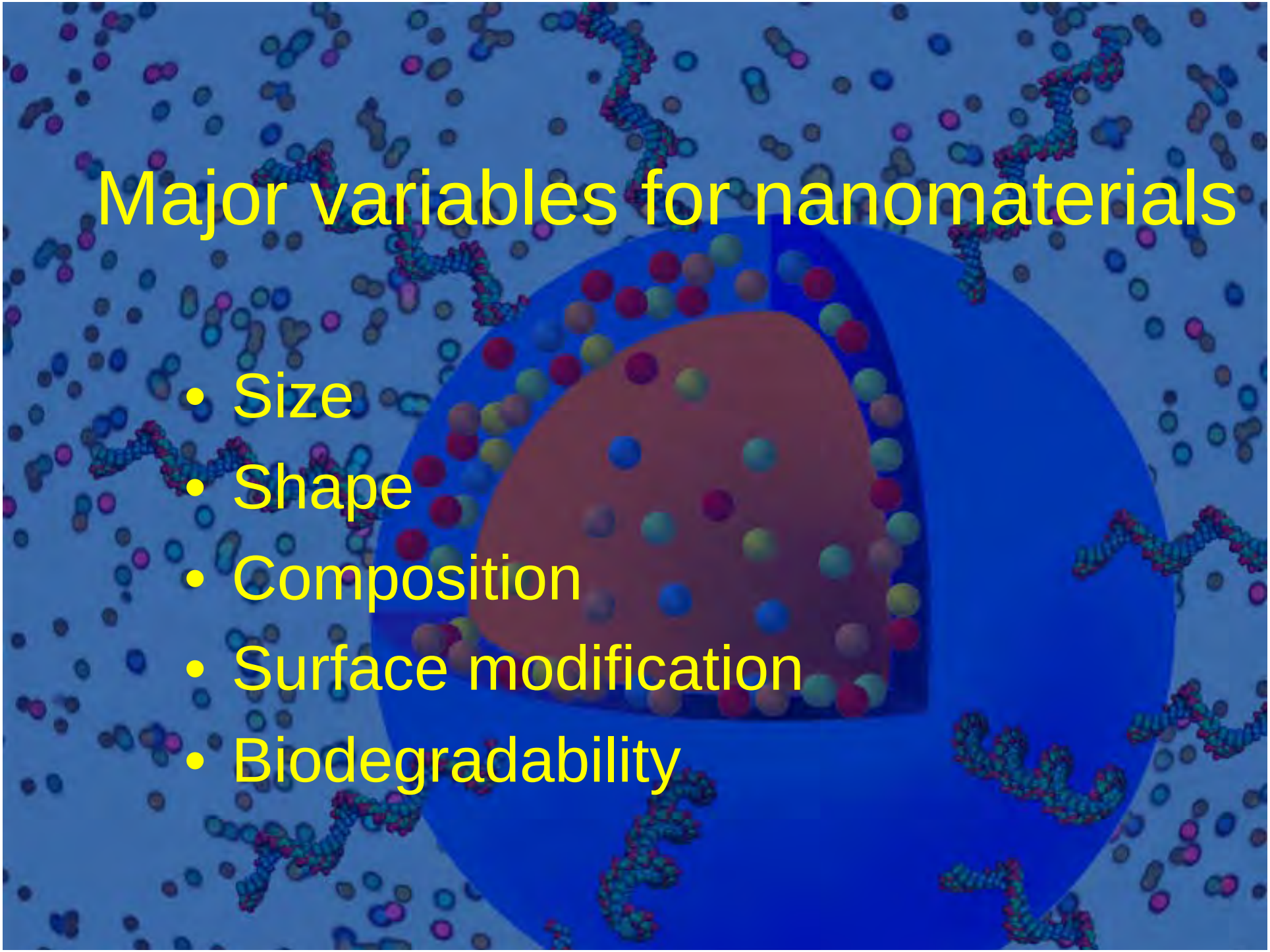
Fullerene Species	Structure	Live Stain	Dead Stain
C_{60}			
C_3			
$Na^{+}_{2-3}[C_{60}O_{7-9}(OH)_{12-15}]^{(2-3)-}$			

Sayes, C.M. Nano Letters 4, 1881

Mechanism - Free radical generation by carbon nanomaterials



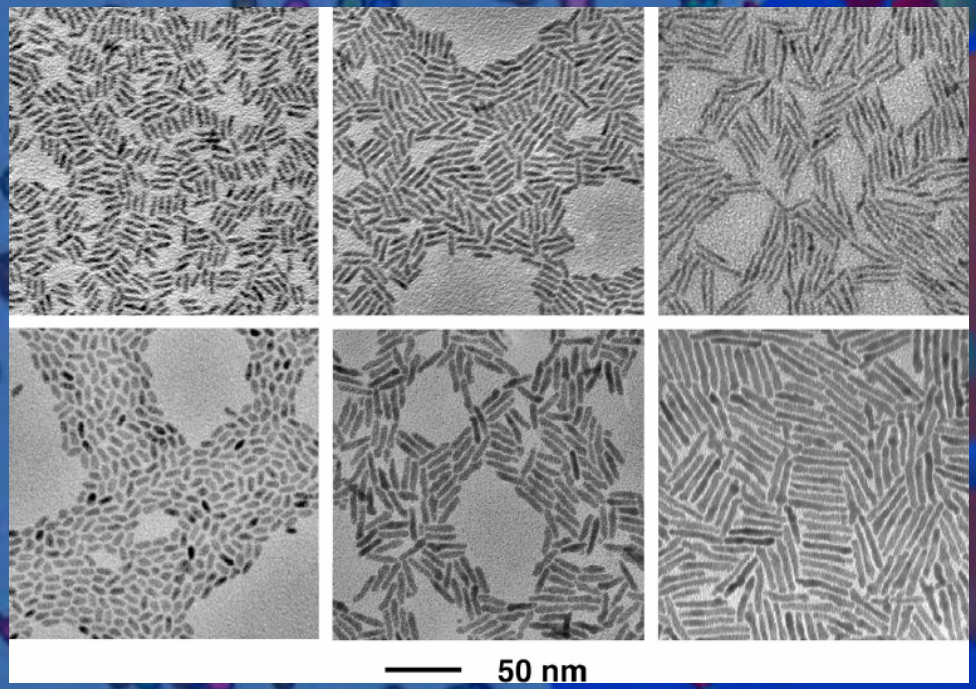
Ali, S.S. et al. Free Radical Biology & Medicine, Vol. 37, No. 8, pp. 1191–1202, 2004

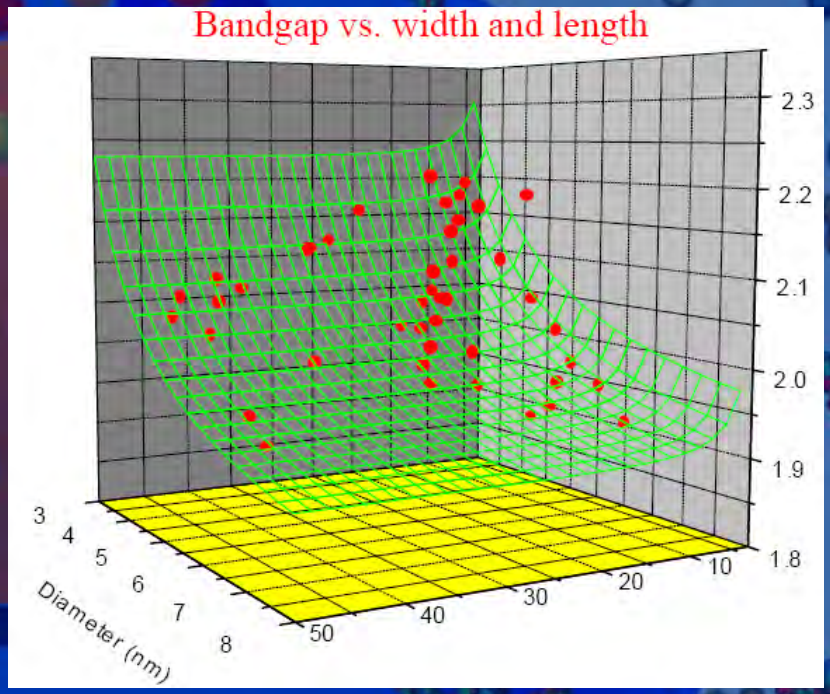


Major variables for nanomaterials

- Size
- Shape
- Composition
- Surface modification
- Biodegradability

Nanomaterial chemical/physical matrix





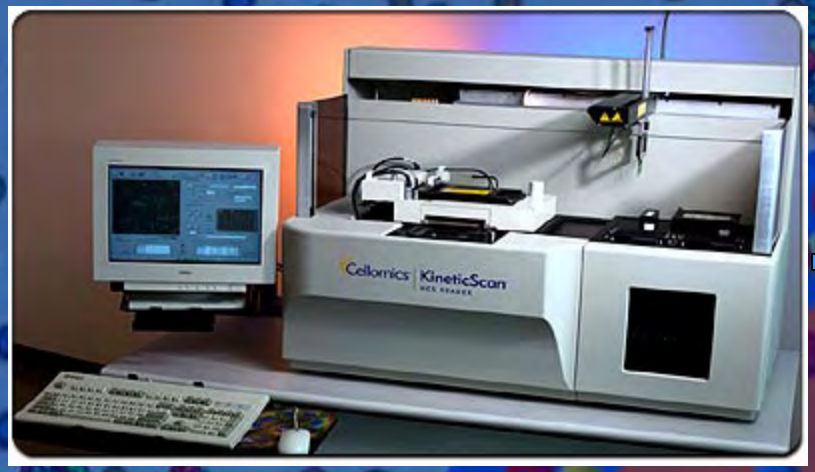
Independent control of length and diameter

Li, L. S., J. T. Hu, W. D. Yang and A. P. Alivisatos (2001). "Band gap variation of size and shape-controlled colloidal CdSe quantum rods." Nano Letters **1**(7): 349-351.

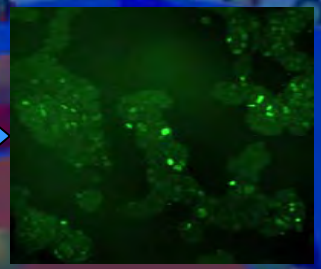
Studies

- Toxigenomic study of nanoparticles (MWCNO, MWCNT, Qdot, Au) using Affy HTA GeneChip microarray
- High content cellomic study of nanoparticle effect on cellular level
- Proteomic profiling
- Metabolomics profiling, isotopomer flux analysis
- Real-time in vivo tracking

High Content Analysis of Pathway Activation/Interference



Relocalization of $p27^{kip}$ to nucleus after treatment with herceptin



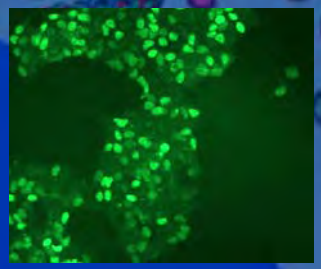


Image analysis performed on thousands of cells to ascertain response

Treat and analyze with Cellomics ArrayScan

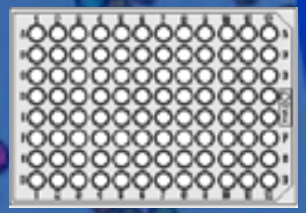
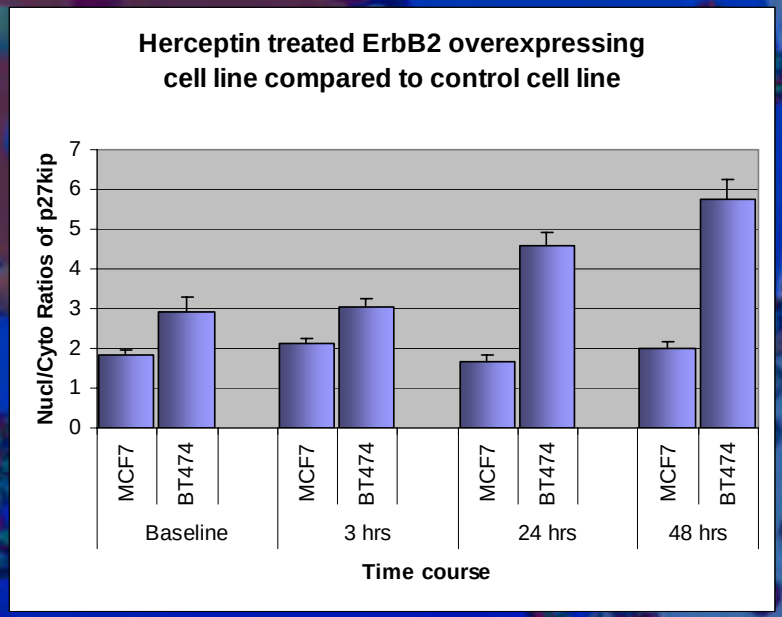


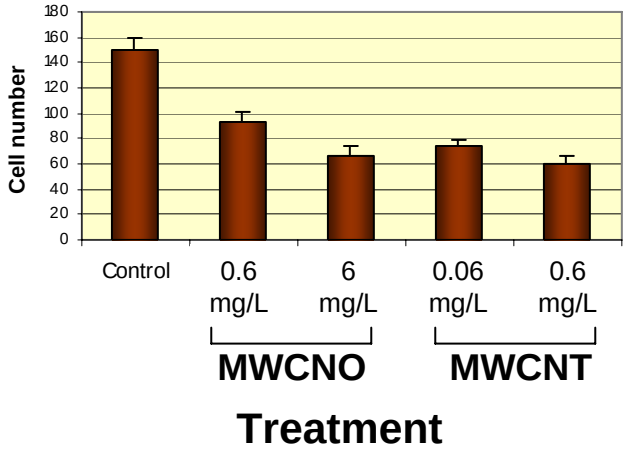
Plate cells on 96-well plate



Apoptosis & Necrosis

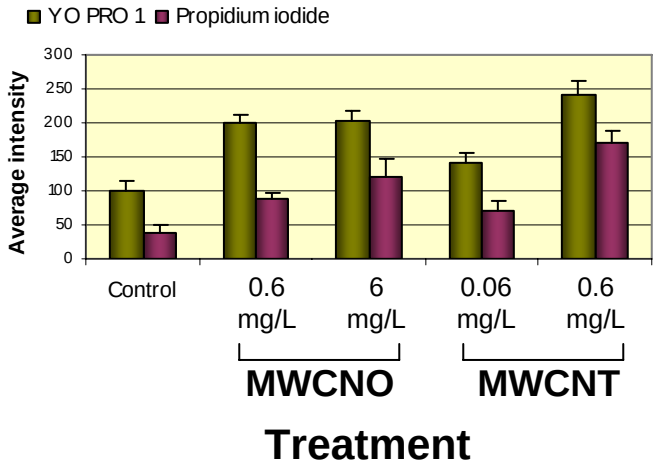
A

Number of Cells



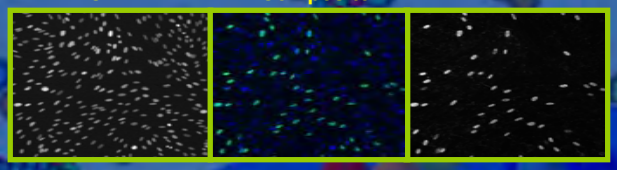
B

Apoptosis and Necrosis



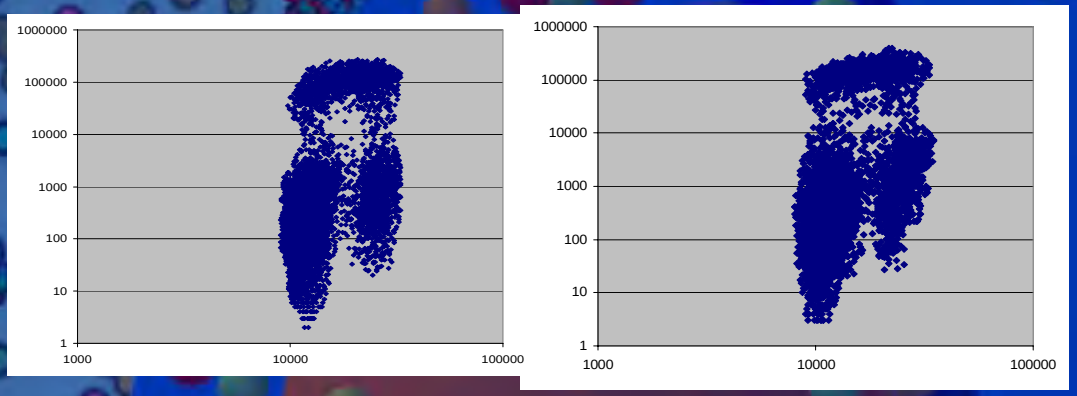
Cell cycle perturbation

a



b

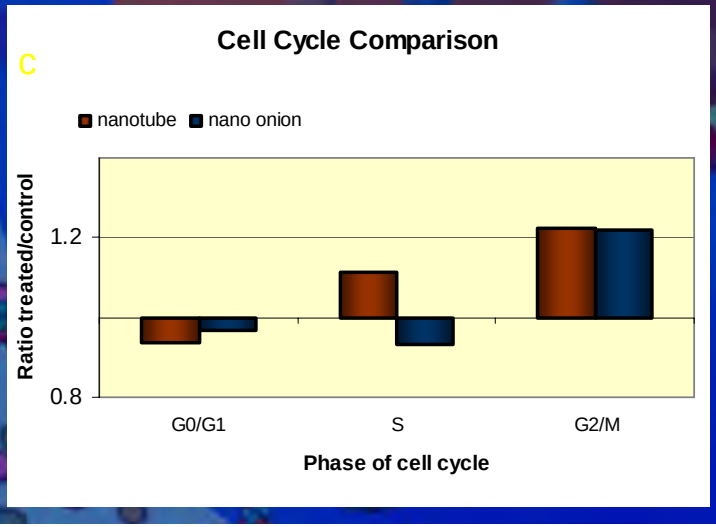
BrdU and PI data obtained from HCS with Cellomics



PI intensity-nanotubes

PI intensity- nanoonions

c



Gene expression profiles

- Genome-wide profiling of the biological effects of nanoparticle from early damage at the molecule level, nanoparticle-induced changes
- Qualitative biomarker discovery, and quantitative biodosimetry for nanoparticle
- Risk assessment and outcome prediction
- Help to develop a model for risk assessment

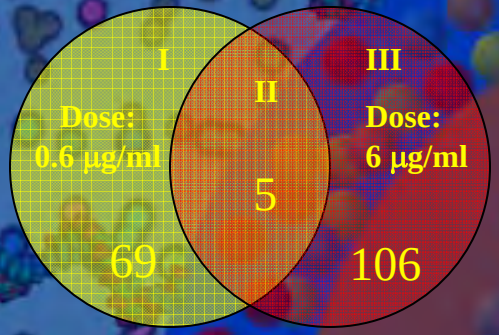
Statistical analysis

- Percentage of differentially expressed genes.
- Principal Component Analysis (PCA)
- Cluster analysis.
- Patterns of expression changes.
- Pathway analysis
- Gene functional group analysis.
- Comparison of gene lists that are induced/suppressed by various experimental conditions (Venn Diagram)

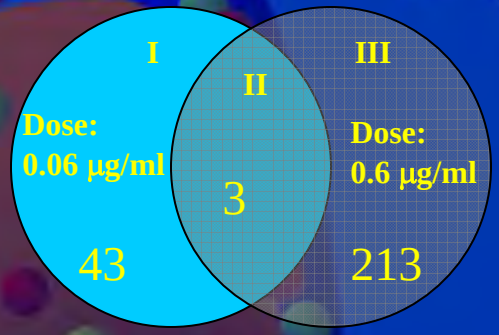
A

Dose	MWCNO		MWCNT	
	Low dose (0.6 μg/ml)	High dose (6 μg/ml)	Low dose (0.06 μg/ml)	High dose (0.6 μg/ml)
Gene number	74	111	46	216

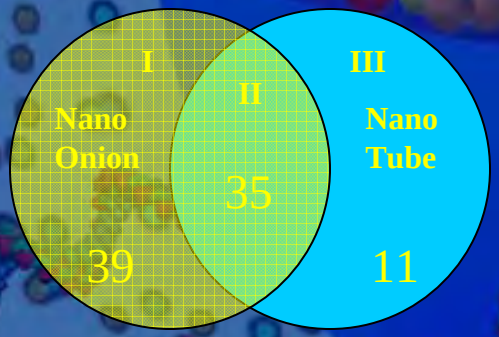
B Carbon Nano-onion



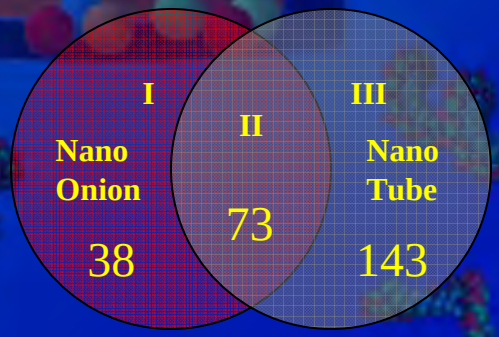
C Carbon Nanotube



D 0.6 μg/ml (MWCNO) vs. 0.06 μg/ml (MWCNT)



E 6 μg/ml (MWCNO) vs. 0.6 μg/ml (MWCNT)



GO category

CarbonTube 0.06 mg/L

Term	P-Value	Percentage of under expressed	Percentage of over expressed	Percentage of changed
Golgi vesicle transport	0.00070	4.26%	2.13%	6.38%
protein metabolism	0.00200	0.65%	0.18%	0.82%
secretory pathway	0.00490	2.17%	1.09%	3.26%
fatty acid biosynthesis	0.00760	5.71%	0.00%	5.71%
G1/S transition of mitotic cell cycle	0.01350	4.26%	0.00%	4.26%
protein ubiquitination	0.01740	0.68%	1.37%	2.05%
mitotic cell cycle	0.02000	1.95%	0.00%	1.95%
ubiquitin cycle	0.02140	0.70%	0.70%	1.41%
cell homeostasis	0.02280	3.23%	0.00%	3.23%
protein prenylation	0.02620	14.29%	0.00%	14.29%

CarbonTube 0.6 mg/L

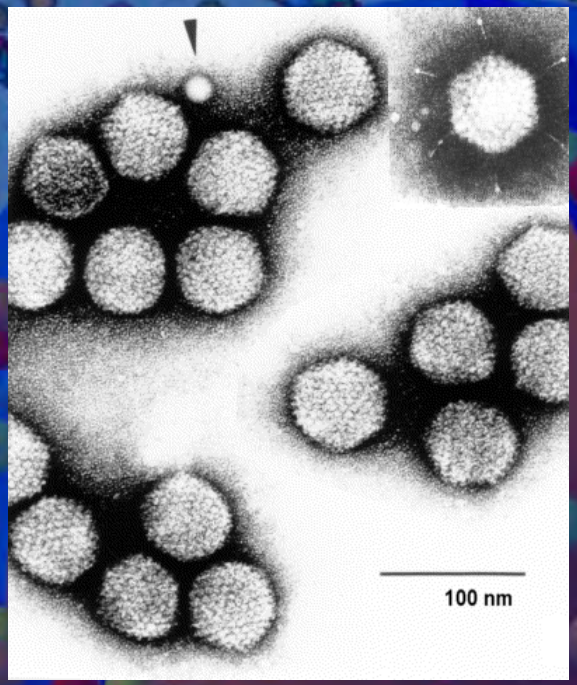
Term	P-Value	Percentage of under expressed	Percentage of over expressed	Percentage of changed
tRNA aminoacylation	0.00000	0.00%	33.33%	33.33%
L-serine metabolism	0.00000	0.00%	50.00%	50.00%
amine metabolism	0.00000	0.00%	6.90%	6.90%
amine transport	0.00000	0.00%	14.63%	14.63%
response to stimulus	0.00000	0.16%	2.86%	3.02%
immune response	0.00000	0.18%	4.50%	4.68%
water-soluble vitamin biosynthesis	0.00240	0.00%	40.00%	40.00%
inflammatory response	0.00340	0.00%	5.06%	5.06%
heterocycle metabolism	0.00620	2.13%	6.38%	8.51%
dicarboxylic acid transport	0.00650	0.00%	25.00%	25.00%

Immune and inflammatory genes

Table 3. Immune-response genes that over- or under-expressed after treating HSF42 cells with 80 μg/ml of carbon nano-tubes.

Gene Symbol	Gene Name	Fold Change[a]
ADAR	adenosine deaminase, RNA-specific	1.44
BDKRB1	bradykinin receptor B1	1.59
CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	1.53
CXCL10	chemokine (C-X-C motif) ligand 10	4.82
CXCL3	chemokine (C-X-C motif) ligand 3	2.71
G1P2	interferon, alpha-inducible protein (clone IFI-15K)	2.51
G1P3	interferon, alpha-inducible protein (clone IFI-6-16)	2.03
IFI44	interferon-induced protein 44	3.50
IFIT1	interferon-induced protein with tetratricopeptide repeats 1	6.99
IFIT2	interferon-induced protein with tetratricopeptide repeats 2	5.99
IFIT3	interferon-induced protein with tetratricopeptide repeats 3	5.85
IFIT5	interferon-induced protein with tetratricopeptide repeats 5	1.76
IRF1	interferon regulatory factor 1	2.02
IRF7	interferon regulatory factor 7	2.47
ISGF3G	interferon-stimulated transcription factor 3, gamma 48kDa	1.55
LIF	leukemia inhibitory factor (cholinergic differentiation factor)	2.67
MGST2	microsomal glutathione S-transferase 2	0.67
MX1	Homo sapiens myxovirus (influenza) resistance 1	11.18
MX2	myxovirus (influenza virus) resistance 2 (mouse)	6.88
NFE2L1	nuclear factor (erythroid-derived 2)-like 1	1.70
NR4A2	nuclear receptor subfamily 4, group A, member 2	3.26
OAS1	2',5'-oligoadenylate synthetase 1, 40/46kDa	2.82
OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa	2.79
OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa	2.21
RIPK2	receptor-interacting serine-threonine kinase 2	1.45
TNFAIP6	tumor necrosis factor, alpha-induced protein 6	1.82

Toxicogenomic study with gene delivery vehicles



High Content Screening of pathways and cellular phenotypes

Cellular transport genes

Gene category	Gene Symbol	Gene Name	Fold change of gene expression for onion 0.6 µg/ml
Golgi vesicle transport	COPA	coatomer protein complex, subunit alpha	0.57
	SNAP23	synaptosomal-associated protein	0.30
	GBF1	golgi-specific brefeldin A resistance factor 1	2.45
	NAPG	N-ethylmaleimide-sensitive factor attachment protein, gamma	0.48
	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.60
membrane fusion	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.60
	NAPG	N-ethylmaleimide-sensitive factor attachment protein, gamma	0.48
	SNAP23	synaptosomal-associated protein	0.30
secretory pathway	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.60
	COPA	coatomer protein complex, subunit alpha	0.57
	GBF1	golgi-specific brefeldin A resistance factor 1	2.45
	NAPG	N-ethylmaleimide-sensitive factor attachment protein, gamma	0.48
	SCD	stearoyl-CoA desaturase	0.19
	SNAP23	synaptosomal-associated protein	0.30
intracellular transport	GBF1	golgi-specific brefeldin A resistance factor 1	2.45
	DST	dystonin	0.40
	NAB2	NGFI-A binding protein 2	0.43
	SNAP23	synaptosomal-associated protein	0.30
	KDELR3	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3	0.76
	NAPG	N-ethylmaleimide-sensitive factor attachment protein, gamma	0.48
	COPA	coatomer protein complex, subunit alpha	0.57
	HNRPA1	heterogeneous nuclear ribonucleoprotein A 1	2.49
	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.60
nucleocytoplasmic transport	NAB2	NGFI-A binding protein 2	0.43
	HNRPA1	heterogeneous nuclear ribonucleoprotein A 1	2.49

Gene category	Gene Symbol	Gene Name	Fold change of gene expression for tube 0.06 µg/ml
Golgi vesicle transport	COPA	coatomer protein complex, subunit alpha	0.57
	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.60
	GBF1	golgi-specific brefeldin A resistance factor 1	2.45
membrane fusion	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.60
secretory pathway	COPA	coatomer protein complex, subunit alpha	0.57
	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.60
	SCD	stearoyl-CoA desaturase	0.19
	GBF1	golgi-specific brefeldin A resistance factor 1	2.45
intracellular transport	GBF1	golgi-specific brefeldin A resistance factor 1	2.45
	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.60
	COPA	coatomer protein complex, subunit alpha	0.57

Cell cycle genes

Gene category	Gene Symbol	Gene Name	Fold change of gene expression for nano-onion
cell proliferation	EXTL3	exostoses (multiple)-like 3	0.44
	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	1.72
	NAB2	NGFI-A binding protein 2 (EGR1 binding protein 2)	0.43
cell cycle	DUSP1	dual specificity phosphatase 1	0.17
	TRIM33	tripartite motif-containing 33	1.60
	HSF1	heat shock transcription factor 1	0.52
	BCAT1	branched chain aminotransferase 1, cytosolic	0.17
regulation of cell cycle	SKP2	S-phase kinase-associated protein 2 (p45)	0.21
	MCL1	myeloid cell leukemia sequence 1 (BCL2-related)	0.19
	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	0.24
	MAPK14	mitogen-activated protein kinase 14	0.25
	CRKL	v-crk sarcoma virus CT10 oncogene homolog (avian)-like	0.20
cell cycle arrest	MACF1	microtubule-actin crosslinking factor 1	1.81
	DST	dystonin	0.40
cell differentiation	PDLIM7	PDZ and LIM domain 7 (enigma)	0.60
	BSG	basigin (OK blood group)	0.44
	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.60
	EGR1	early growth response 1	0.39

Gene category	Gene Symbol	Gene Name	Fold change of gene expression for nanotube
cell proliferation	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	1.72
cell cycle	DUSP1	dual specificity phosphatase 1	0.23
	BCAT1	branched chain aminotransferase 1, cytosolic	0.24
	CDK2	cyclin-dependent kinase 2	0.58
regulation of cell cycle	SKP2	S-phase kinase-associated protein 2 (p45)	0.18
	MCL1	myeloid cell leukemia sequence 1 (BCL2-related)	0.18
	MAPK14	mitogen-activated protein kinase 14	0.30
	CRK	v-crk sarcoma virus CT10 oncogene homolog (avian)	0.53
	SLC12A4	solute carrier family 12 (potassium/chloride transporters), member 4	0.23
cell differentiation	PDLIM7	PDZ and LIM domain 7 (enigma)	0.62
	NAPA	N-ethylmaleimide sensitive fusion protein attachment protein alpha	0.62

Apoptosis

Gene symbol	Gene Name	Fold change of gene expression for 0.6 µg/ml onion
EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	0.17
MCL1	myeloid cell leukemia sequence 1 (BCL2-related)	0.19
BCL2L1	BCL2-like 1	0.24
PPM1F	protein phosphatase 1F (PP2C domain containing)	1.63
TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	0.35
FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	1.72
CRKL	v-crk sarcoma virus CT10 oncogene homolog (avian)-like	0.20
EXTL3	exostoses (multiple)-like 3	0.44
MAPK14	mitogen-activated protein kinase 14	0.31
MACF1	microtubule-actin crosslinking factor 1	1.81

Gene symbol	Gene Name	Fold change of gene expression for 0.06 µg/ml tube
TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	0.40
MCL1	myeloid cell leukemia sequence 1 (BCL2-related)	0.18
FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	1.72
CRK	v-crk sarcoma virus CT10 oncogene homolog (avian)	0.48
MAPK14	mitogen-activated protein kinase 14	0.30

Gene symbol	Gene Name	Fold change of gene expression for 6 µg/ml onion
YARS	tyrosyl-tRNA synthetase	1.62

Gene symbol	Gene Name	Fold change of gene expression for 0.6 µg/ml tube
YARS	tyrosyl-tRNA synthetase	1.75
MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	11.55
BIRC3	baculoviral IAP repeat-containing 3	2.16
RIPK2	receptor-interacting serine-threonine kinase 2	1.38
STAT1	signal transducer and activator of transcription 1, 91kDa	2.22
TNFAIP3	tumor necrosis factor, alpha-induced protein 3	1.95
AHR	aryl hydrocarbon receptor	1.68
TNFRSF10B	tumor necrosis factor receptor superfamily, member 10b	1.62

Stimuli

Gene Category	Gene symbol	Gene Name	MWCNO 6mg/ml
immune response	EGR1	early growth response 1	0.37
	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	0.14
Stress response	DDIT3	DNA-damage-inducible transcript 3	2.39
	SLC3A2	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	2.46
	STC2	stanniocalcin 2	2.38
	VEGF	vascular endothelial growth factor	2.18
	DDIT3	DNA-damage-inducible transcript 3	2.39
	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	0.14
	SQSTM1	sequestosome 1	2.00
	VEGF	vascular endothelial growth factor	2.18

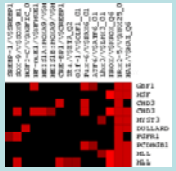
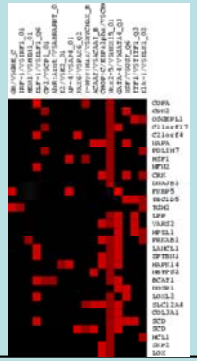
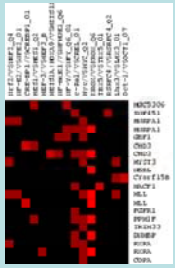
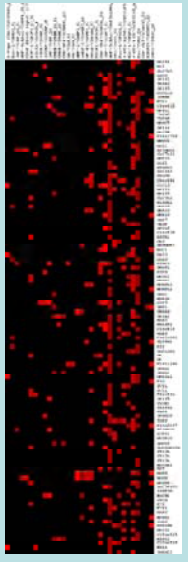
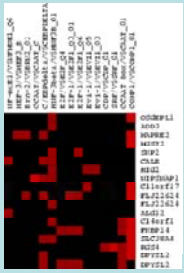
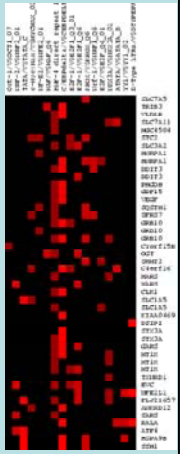
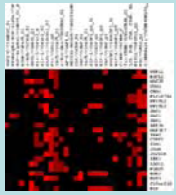
Gene Category	Gene symbol	Gene Name	MWCNT 0.6mg/ml
immune response	CXCL10	chemokine (C-X-C motif) ligand 10	4.82
	IFIT2	interferon-induced protein with tetratricopeptide repeats 2	5.99
	IFIT3	interferon-induced protein with tetratricopeptide repeats 3	5.85
	IRF1	interferon regulatory factor 1	2.02
	IRF7	interferon regulatory factor 7	2.47
	CXCL3	chemokine (C-X-C motif) ligand 3	2.71
	MX2	myxovirus (influenza virus) resistance 2 (mouse)	6.88
	NR4A2	nuclear receptor subfamily 4, group A, member 2	2.62
	PLSCR1	phospholipid scramblase 1	2.38
response to DNA damage stimulus	DDIT3	DNA-damage-inducible transcript 3	2.70
	IRF7	interferon regulatory factor 7	2.47
Stress response	CXCL10	chemokine (C-X-C motif) ligand 10	4.82
	CXCL3	chemokine (C-X-C motif) ligand 3	2.71
	DDIT3	DNA-damage-inducible transcript 3	2.70
	IRF7	interferon regulatory factor 7	2.47
	MKNK2	MAP kinase interacting serine/threonine kinase 2	2.11
	MX2	myxovirus (influenza virus) resistance 2 (mouse)	6.88
	NR4A2	nuclear receptor subfamily 4, group A, member 2	2.62
	OAS1	2',5'-oligoadenylate synthetase 1, 40/46kDa	2.82
	OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa	2.79
	OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa	2.21
	PLSCR1	phospholipid scramblase 1	2.38
	SLC3A2	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	2.58
	STAT1	signal transducer and activator of transcription 1, 91kDa	2.22
	VEGF	vascular endothelial growth factor	2.24

Ubiquitination genes

Gene Category	Gene symbol	Gene Name	Fold change of gene expression MWCNO 0.6 μg/ml
Ubiquitination	CHD3	chromodomain helicase DNA binding protein 3	2.27
	MYST3	MYST histone acetyltransferase (monocytic leukemia)	2.01
	SKP2	S-phase kinase-associated protein 2 (p45)	0.21
	TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	0.35
	TRIM33	tripartite motif-containing 33	1.60

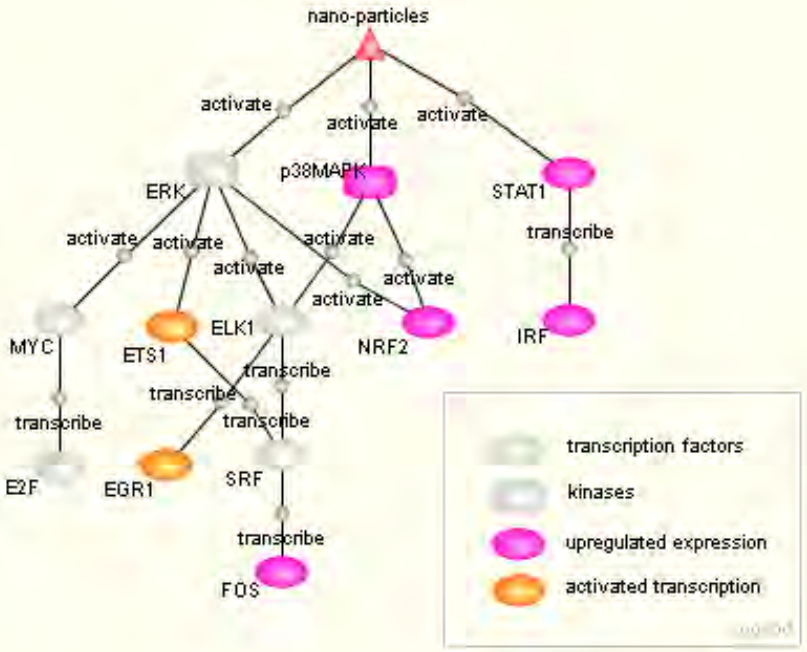
Gene Category	Gene symbol	Gene Name	Fold change of gene expression MWCNT 0.06 μg/ml
Ubiquitination	TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	0.40
	MYST3	MYST histone acetyltransferase (monocytic leukemia)	2.10
	SKP2	S-phase kinase-associated protein 2 (p45)	0.18
	CHD3	chromodomain helicase DNA binding protein 3	2.20

Promoter analysis

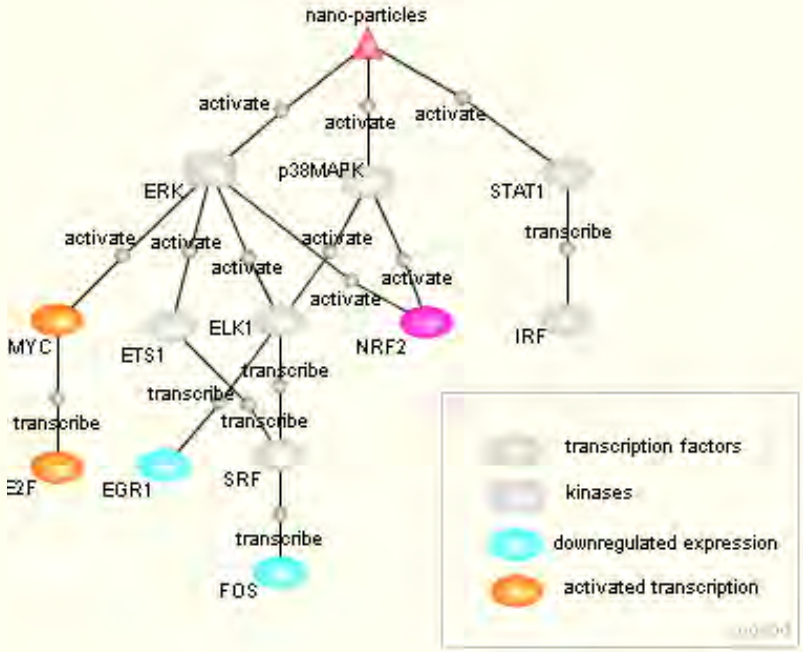
	Multiwall carbon nanotube		Multiwall carbon nano-onion	
	Up-regulated	**Down-regulated**	**Up-regulated**	**Down-regulated**
Low doses				
	Krox	GATA-4, USF, elk-1,	Krox	GATA4, elk1, USF, Krox
High doses				
	IRF, IRF-7, c-ets1, Krox	COMP1	C/EBP-delta, E2F1,Krox	GATA1, HES1, PAX,E2F1

Comparison of pathways networks

A. Response to MWCNT treatment



B. Response to carbon nano-onion treatment



a

2nm DNA alpha helix

4nm Globular protein

6nm microfilaments

11nm Ribosome

25nm microtubule

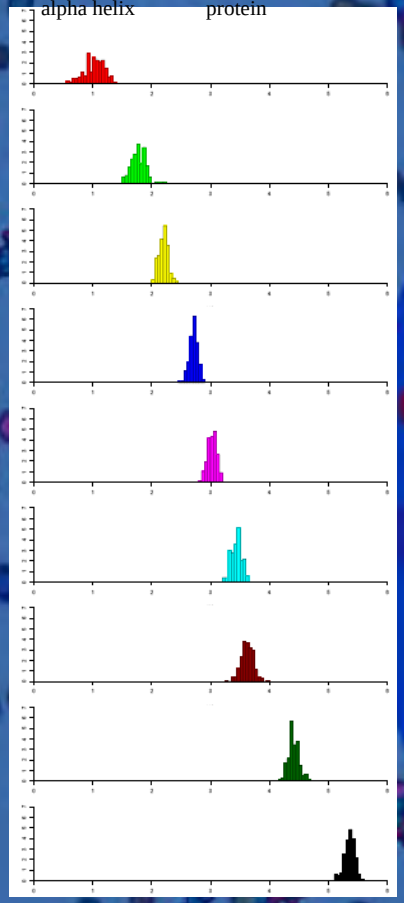
50nm Nuclear pore

100nm Large virus

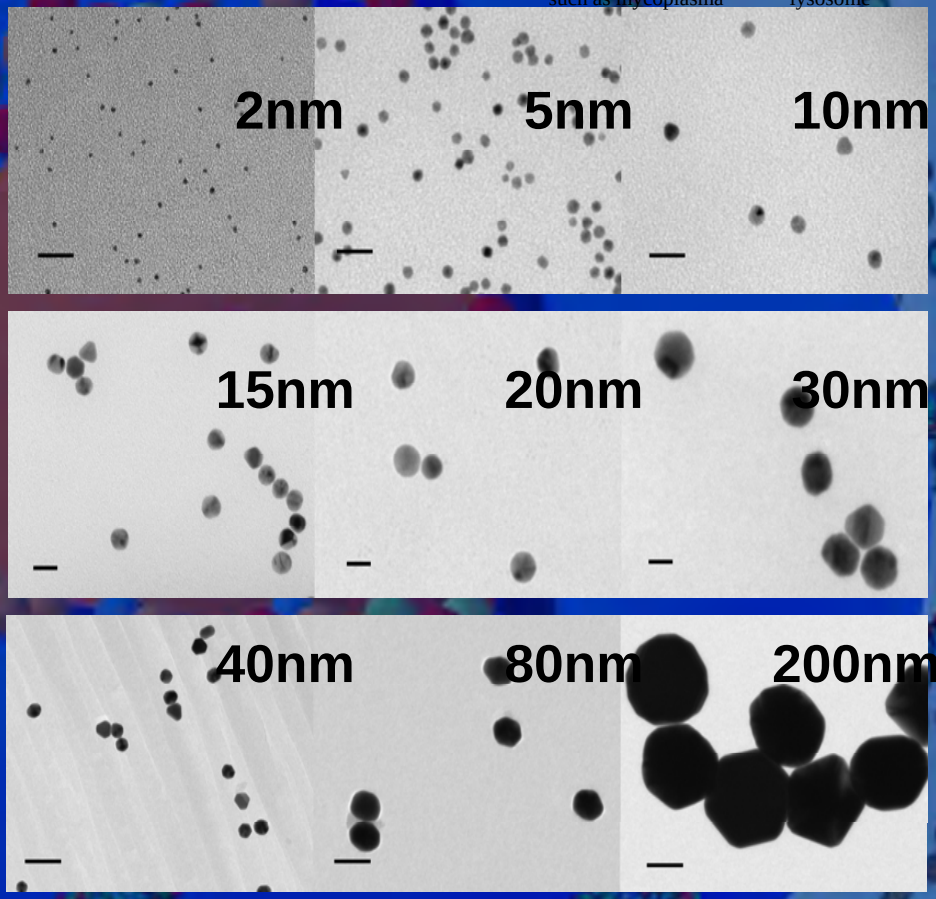
150-250nm Small bacteria such as mycoplasma

200-500nm Peroxisome lysosome

b



c



Scale bar: 20nm for 2nm-30nm, 100nm for 40-200nm

Figure 2. Apoptosis and Gene Expression UP- and Down-regulation

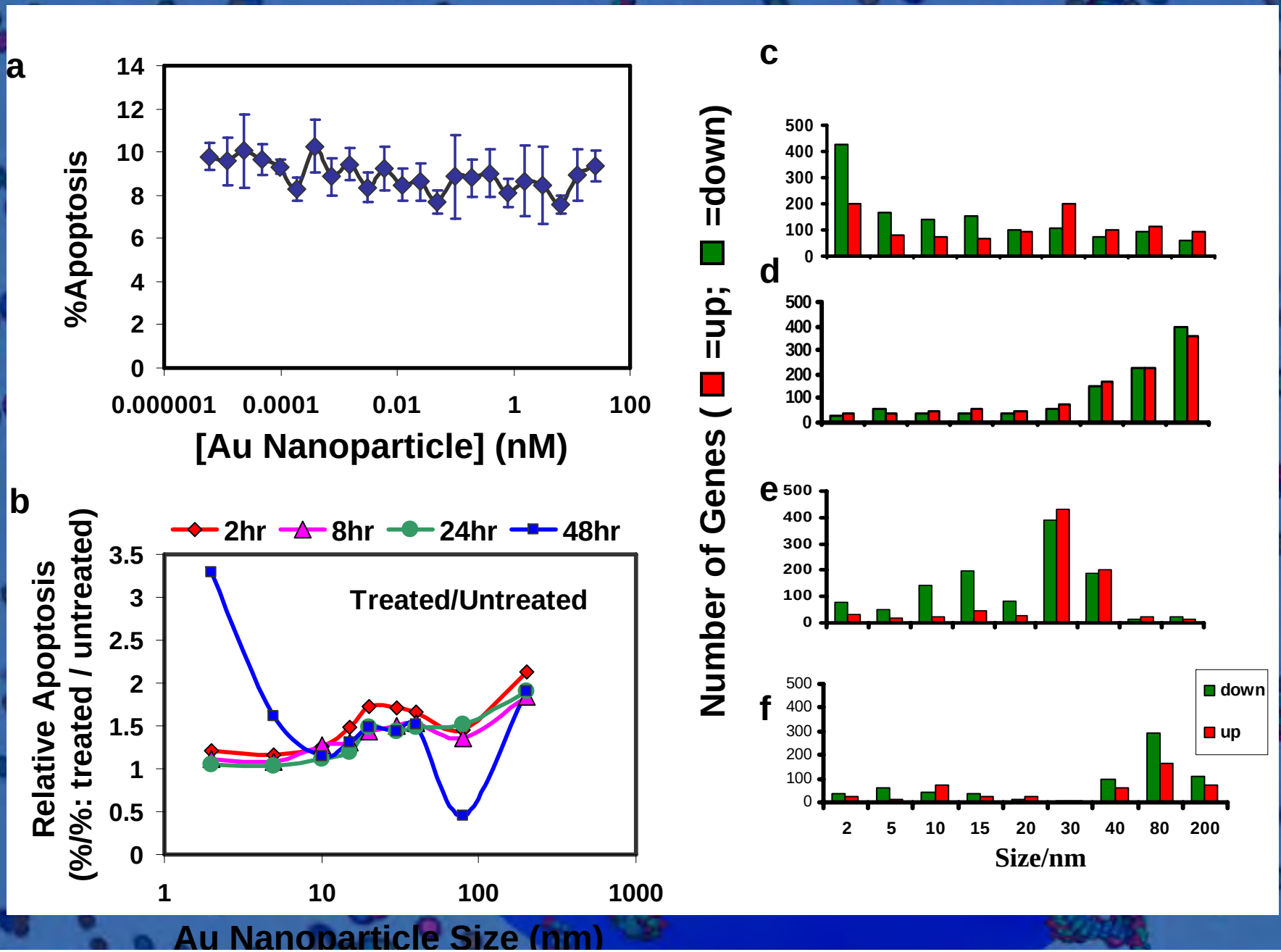
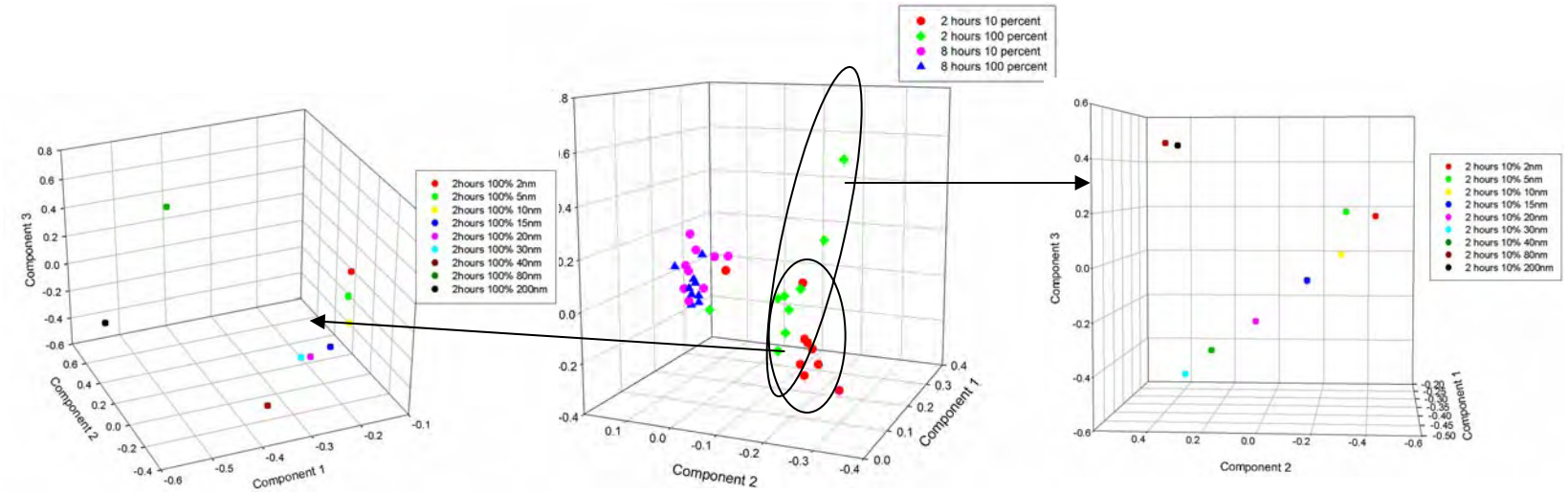


Figure 3. PCA Analysis



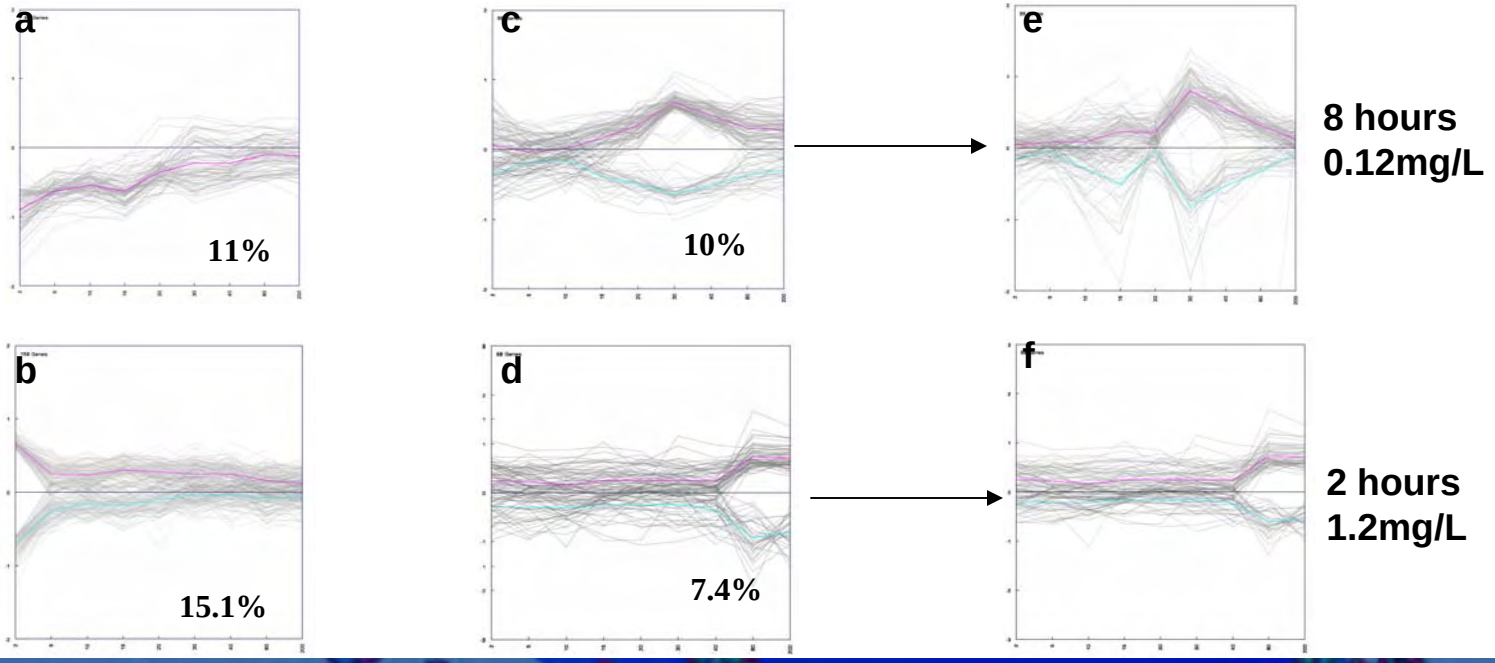
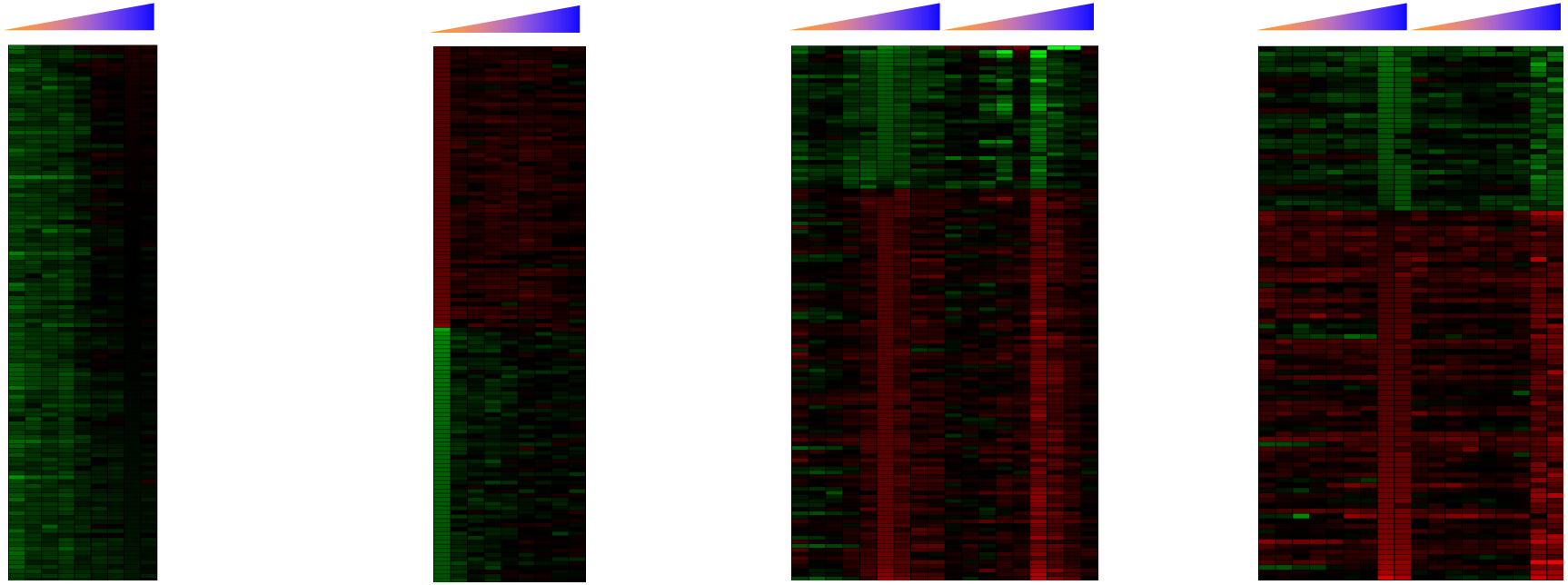


Figure 4. Heatmap and Clustering



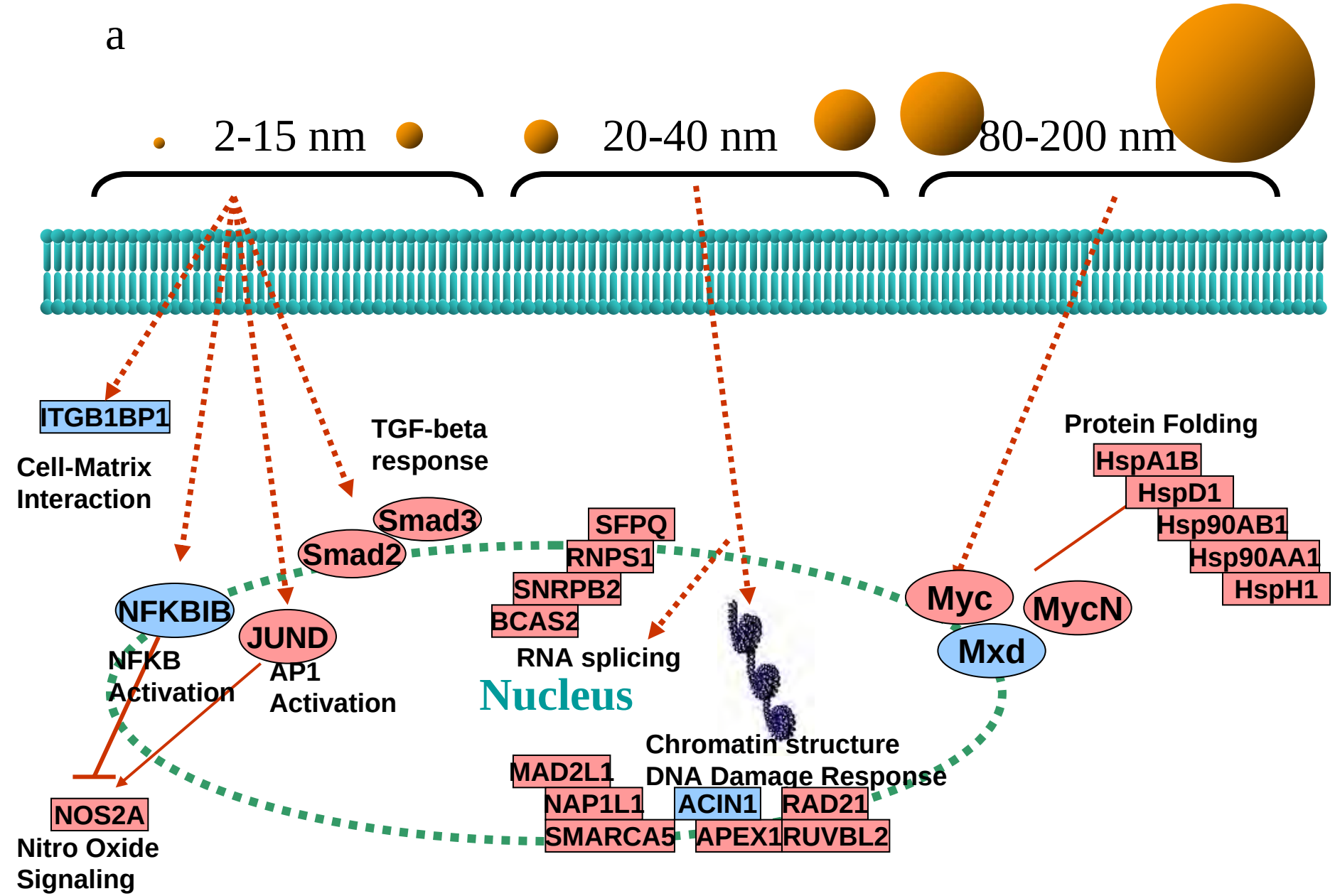
Cytoskeleton organization and biogenesis	TMSB4X SCIN NCKIPSD CAPZA1
Transcription, DNA-dependent	NFATC3 NMI BLZF1 ZNF33A AIRE ZNF623 POLR2B VHL TTF2 ZNF549 AHR ZNF14 ZNF417 MCM8
DNA repair	RAD23A XRCC2 NTHL1
Secretion	BLZF1 ICA1
Response to stress	IL18 NFATC3 NMI AIRE RAD23A VHL GRAP2 XRCC2 NTHL1 AHR

Transcription	BHLHB2, ESR2, FOXD1, GTF2B, GTF2IRD1, JUND, SIRT2, SMAD2, SMAD3, SUB1, YBX1, YWHAH
Growth	CA9, DVL1, EIF5A2, ESR2, EWSR1, JUND, NFKBIB, NOS2A, PCSK4, RAB1A, SMAD2, SMAD3, TRA2A, ZNF198
Cell Signaling	CEP57, DVL1, ESR2, FASTK, GABRB3, GABRQ, NFKBIB, NOS2A, PDE1B, PDGFC, PRKRIR, SH3GL3, SMAD2, SMAD3, STXBP4, YWHAH
Activation of virus	GTF2B, JUND, SMAD3, SUB1
Apoptosis	DUSP6, DVL1, NOS2A
Transport of protein	HSPA9B RAB10, RAB1A, RAB6A, YWHAH

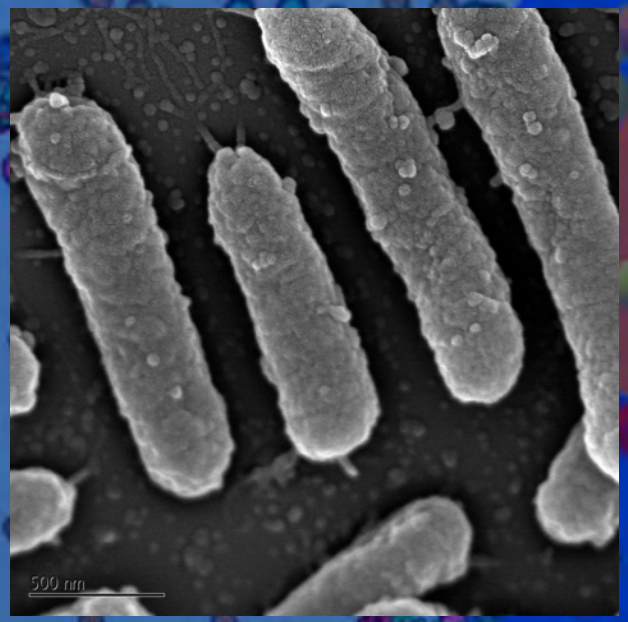
Chromosome organization and biogenesis	RAD21 GAS41 RUVBL2 JJAZ1 ACINUS TAF6L NAP1L1 H2AV SMARCA5
DNA repair	APEX1 RAD21 RUVBL2 USP1 SFPQ
DNA packaging	GAS41 RUVBL2 JJAZ1 TAF6L NAP1L1 H2AV HAT1 SMARCA5
Intracellular signaling cascade	CSK HIP14 FKBP1A LZTFL1 HIP-55 SNX16 RAP2C
RNA metabolism	LSM5 BCAS2 RNPS1 SFPQ HNRPH3 NXT2 KHDRBS1 SNRPB2
Transcription, DNA-dependent	HIF1A ZNRD1 APEX1 GAS41 SAP30 RUVBL2 JJAZ1 ZNF146 TAF6L SYBL1 SFPQ NR2F2 VPS4B KHDRBS1 ILF3 SMARCA5 SP3

response to stress	DNAJB1 HSPD1 HSPA8 HMGB2 PTTG2 KIR3DL3 HSPH1 HSPCB DNAJA1 HSPE1
Organismal physiological process	CD1E BMP4 ELA3A ELOVL4 KIR3DL3 RGS16 FCGR1A
Cell cycle	MYC PDGFA PTTG2 ATF5 CCNB2 AURKB
Response to unfolded protein	DNAJB1 HSPD1 HSPA8 HSPH1 HSPCB DNAJA1 HSPE1
Transport	TIRP HSPD1 FTL ETFB FCGR1A
Transcription	C20orf97 MYC MYCN ZNF90 HMGB2 MXD3 PTTG2 ATF5 LRRFIP1

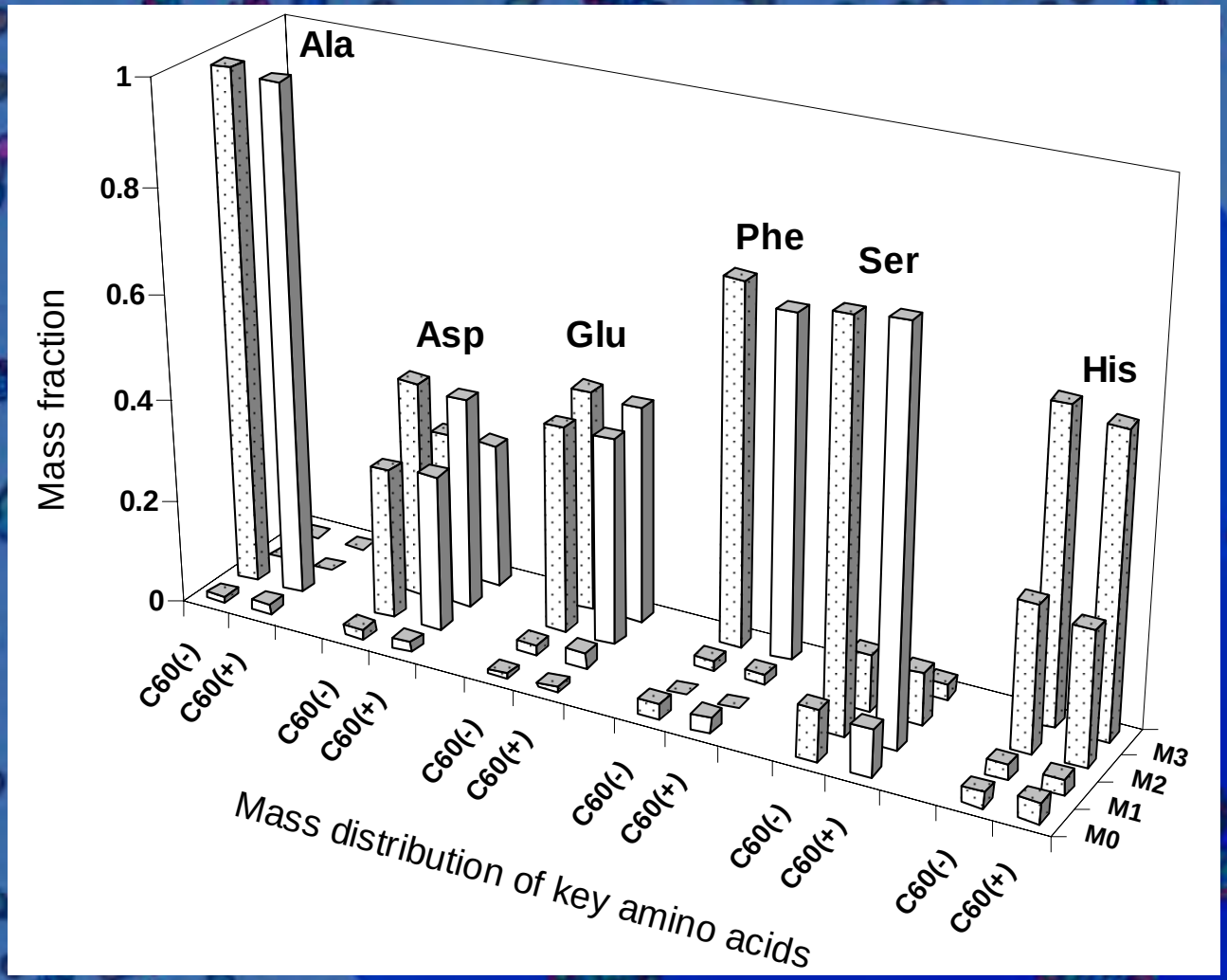
Fig. 5. Pathway Analysis



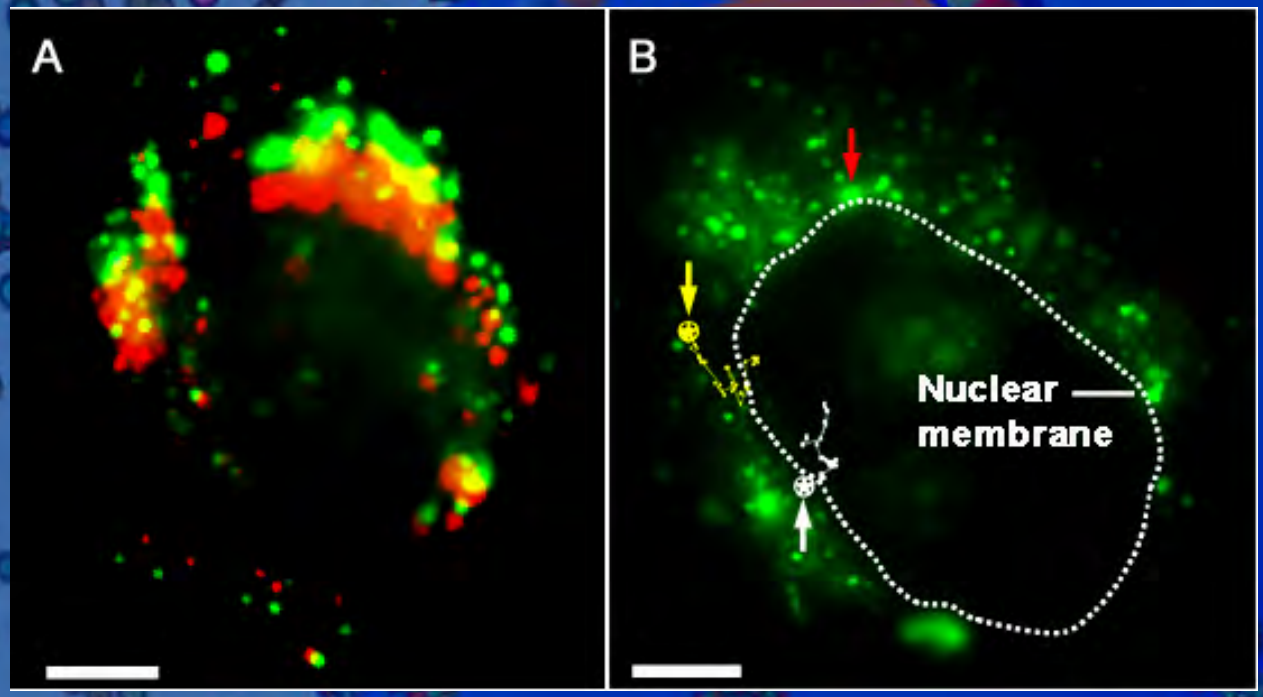
Effect on microbes



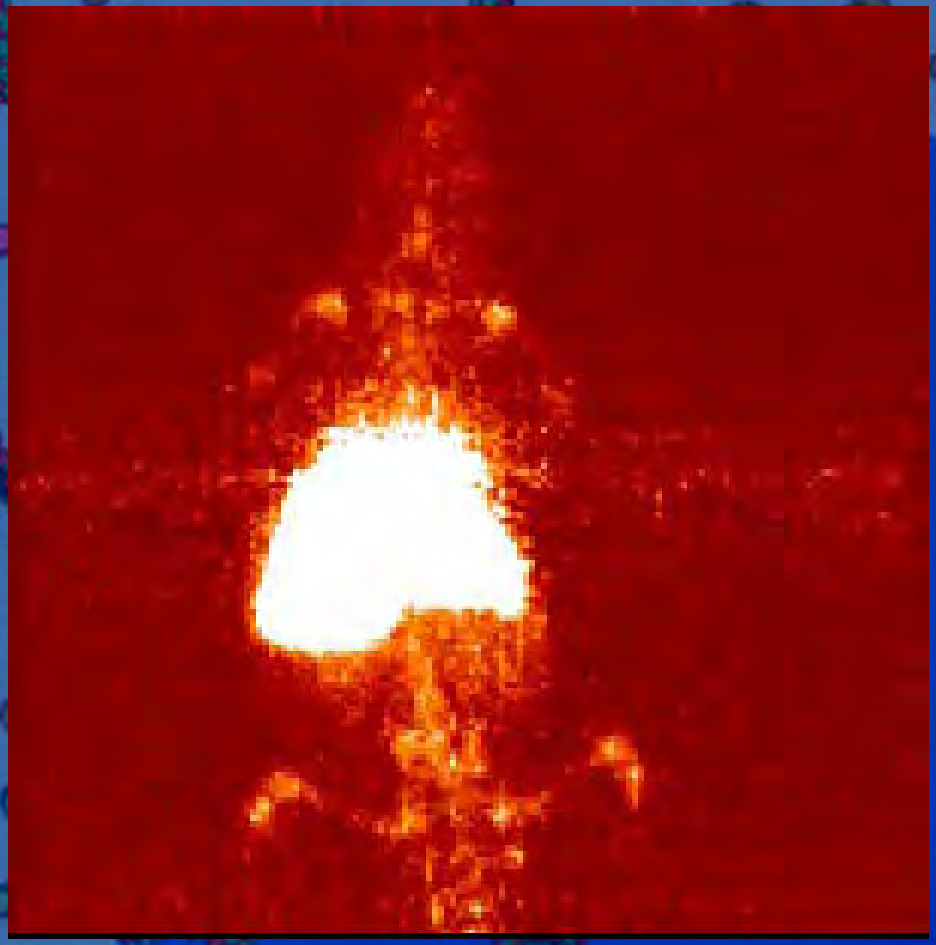
Metabolomic profiling



Time lapse high content microscopy



Nanoparticle in vivo PET imaging



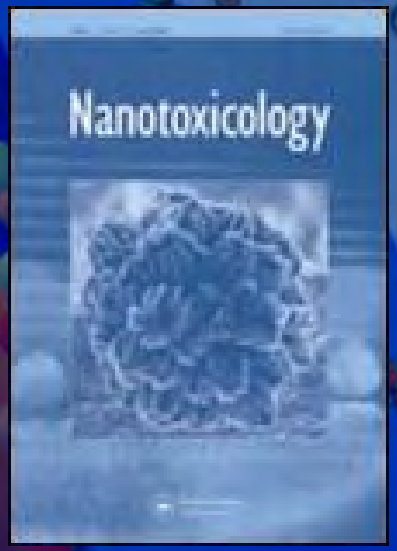
MIP image of biodistribution of ^{64}Cu-quantum dots

Summary

- Pathways and analysis by examination of cellular gene expression (Affy) and high content imaging analysis
- Pathways of cellular transport, apoptosis, cell cycle, ubiquitination, stress response, etc.
- NT is more toxic than Nano-onion
- Response similar to virus
- Size and surface dependent molecular profiles discovered

Nanotoxicology

- Launching in 2007
- www.nanotoxicology.net
- Taylor & Francis



Acknowledgement

- Paul Alivisatos
- Joe Gray
- Yinjie Tang
- Jay Keasling
- Tingting Zhang
- Daniele Gerion
- Jackie Stilwell
- Lianghao Ding
- Omeed Elboudwarej
- Guangwei Min
- John Selegue